Jay Shankar Singh
Gamini Seneviratne *Editors*

Agro-Environmental Sustainability

Volume 2: Managing Environmental Pollution

Springer

Agro-Environmental Sustainability

Jay Shankar Singh • Gamini Seneviratne
Editors

Agro-Environmental Sustainability

Volume 2: Managing Environmental Pollution

Springer

Editors
Jay Shankar Singh
Department of Environmental Microbiology
Babashaeb Bhimrao Ambedkar (Central) University
Lucknow, Uttar Pradesh, India

Gamini Seneviratne
Microbial Biotechnology Unit
National Institute of Fundamental Studies
Kandy, Sri Lanka

ISBN 978-3-319-49726-6 ISBN 978-3-319-49727-3 (eBook)
DOI 10.1007/978-3-319-49727-3

Library of Congress Control Number: 2017930266

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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.

Prof. Panjab Singh
National Academy of Agricultural Sciences (NAAS),
NASC Complex, DPS Marg, Pusa, New Delhi, India

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 — Jay Shankar Singh
Kandy, Sri Lanka — Gamini Seneviratne

Contents

Contributors

Waqar Ahmad Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Muhammad Ahmed Akram Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Hafiz Naeem Asghar Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Siddharth Boudh Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

William Clarke Centre for Solid Waste Bioprocessing, School of Civil Engineering, School of Chemical Engineering, The University of Queensland, St. Lucia, Brisbane, QLD, Australia

Anand K. Gupta Division of Agronomy and Soil Science, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India

Obulisamy Parthiba Karthikeyan Sino-Forest Applied Research Centre for Pearl River Delta Environment, Hong Kong Baptist University, Hong Kong, China

College of Marine and Environmental Sciences, James Cook University, Townsville, QLD, Australia

Sumit Kaushal Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

Muhammad Yahya Khan Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

University of Agriculture, Faisalabad, Vehari, Pakistan

Irina K. Kravchenko Laboratory of Microbial Survival, Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

Arun Kumar Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

HMSP Madawala Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka

Muhammad Naveed Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Vidya Dhar Pandey Department of Botany, Government Post-Graduate College, Rishikesh, Dehradun, Uttarakhand, India

Umesh Pankaj Division of Agronomy and Soil Science, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India

Muhammad Usman Saleem Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Muhammad Saleem Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Mihiri Seneviratne Department of Botany, Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka

Gamini Seneviratne Microbial Biotechnology Unit, National Institute of Fundamental Studies, Kandy, Sri Lanka

Chhatarpal Singh Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

D.P. Singh Department of Environmental Science, School for Environmental Sciences, B.B. Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

Jay Shankar Singh Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

Peter James Strong Centre for Solid Waste Bioprocessing, School of Civil Engineering, School of Chemical, Engineering, The University of Queensland, St. Lucia, Brisbane, QLD, Australia

Shashank Tiwari Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

Pragya Trivedi Division of Agronomy and Soil Science, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India

Rajesh Kumar Verma Division of Agronomy and Soil Science, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India

Sanjeet K. Verma Division of Agronomy and Soil Science, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India

Meththika Vithanage Chemical and Environmental Systems Modelling Research Group, National Institute of Fundamental Studies, Kandy, Sri Lanka

Sandamali Wijesekara Department of Botany, Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka

Weixiang Wu Institute of Environmental Science and Technology, Zhejiang University, Hangzhou, China

Zahir Ahmad Zahir Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Jing Zhu Institute of Environmental Science and Technology, Zhejiang University, Hangzhou, China

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

J.S. Singh (✉)
Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@yahoo.co.in

D.P. Singh
Department of Environmental Science, School for Environmental Sciences, B.B. Ambedkar (Central) University, Raibarelly Road, Lucknow, Uttar Pradesh 226025, India

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_1

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-

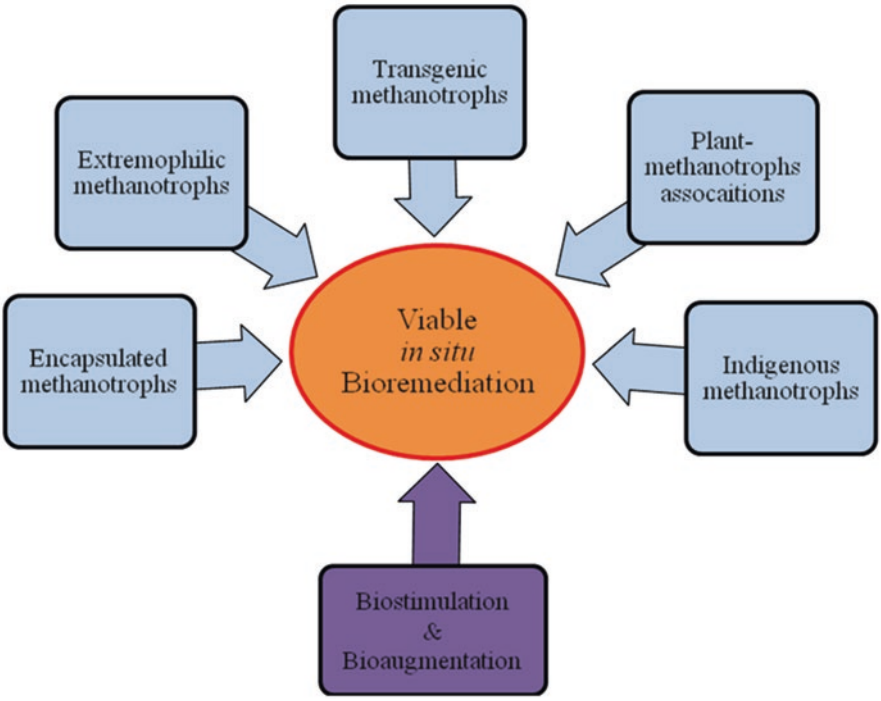


Fig. 1.1 A proposed diagram with different factors that can enhance the in situ bioremediation by methanotrophs

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

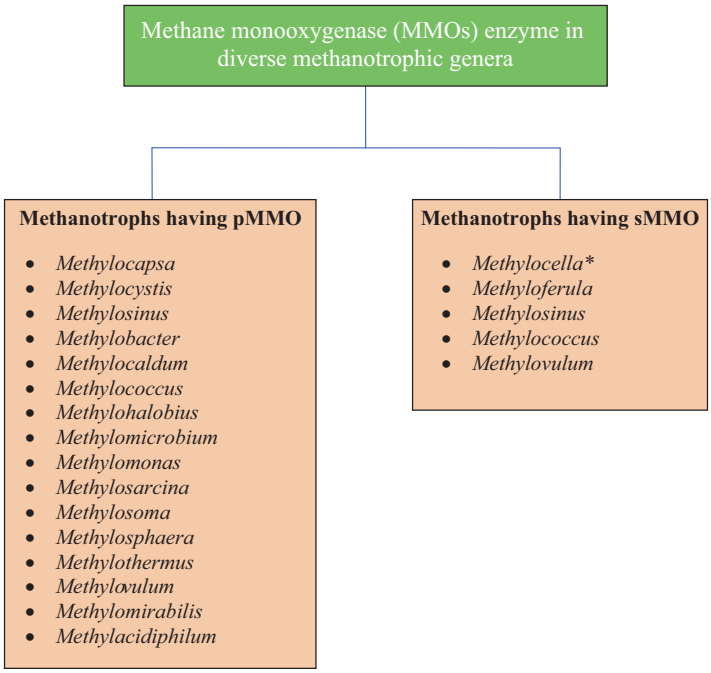


Fig. 1.2 Distribution of pMMO and sMMO among different know methanotrophic genera. *Some *Methylocella* spp. expresses sMMO exclusively

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 CH_4 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
Methylosinus trichosporium OB3b	In laboratory	Halogenated hydrocarbons	Hanson et al. (1990)
			Oldenhuis et al. (1991)
Methylomonas albus BG8, *Methylocystis parvus* OBBP and *Methylosinus trichosporium* OB3b	Aquifer material	Polynuclear aromatic hydrocarbons and transition metals	Jenkins et al. (1994)
Methylosinus trichosporium OB3b	In laboratory	TCE	Lontoh and Semrau (1998)
Type II methanotrophs	Marine enrichment culture	Phenanthrene, Anthracene and Fluorene	Rockne et al. (1998)
Methylocystis sp. M, *Methylococcus capsulatus* (Bath), *Methylosinus trichosporium* OB3b, *Methylosinus sporium* 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)
Methylosinus trichosporium OB3b and *Methylocystis daltona* SB2	In laboratory	TCE, DCE and VC	Yoon (2010)
Methylocystis strain SB2	In laboratory	Vinyl chloride (VC), dichloroethylene (DCE), trichloroethylene (TCE) and chloroform (CF)	Im and Semrau (2001)
Methylophilus methylotrophus EHg7	Industrially contaminated soil	Cadmium (Cd)	De Marco et al. (2004)
Methylophilus methylotrophus ECr4	Industrially contaminated soil	Chromium (Cr)	De Marco et al. (2004)
Methylococcus capsulatus 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). CH_4 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 Cr^{6+}). 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

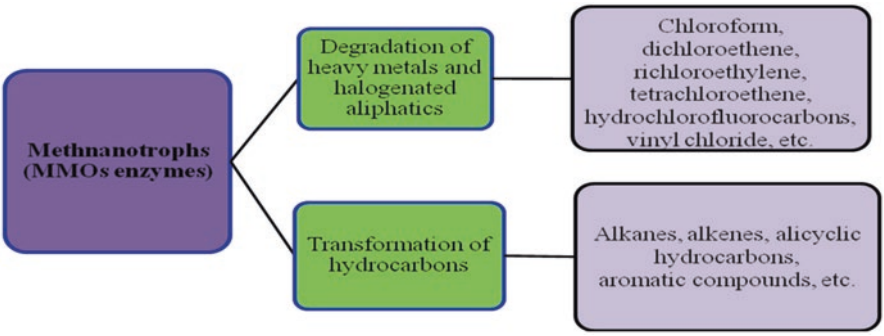


Fig. 1.3 Role of different methanotrophic MMOs enzymes in bioremediation of inorganic and organic pollutants

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 Murrel 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 Murrel 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

may be considered a source of biopolymers and colloids, both of which facilitate the transport of organic hydrocarbons in a hydrophobic environment. This property of methanotrophs may be exploited to accelerate the removal and biodegradation of hydrophobic toxicants. Further study is needed to better understand the ecological and environmental ramifications of exogenous stimulation of indigenous methanotrophs population and their interactions with organic and inorganic pollutants (Jenkins et al. 1994). Many reports have shown that compatible and mixed microbial populations with overall broad base enzymatic capability are required to degrade complex mixtures of hydrocarbons such as crude oil in soil, fresh water and marine environments (Das and Mukherjee 2007; Throne-Holst et al. 2007; Yakimov et al. 2007; Brooijmans et al. 2009). Type II alpha proteobacteria methanotrophs are capable of a wide range of co-metabolic transformations of complex hydrocarbons, and this activity has been exploited in many terrestrial bioremediation systems (Rockne and Strand 2003). Methanotrophic bacteria have been isolated from marine sediments which exhibited ability to biodegrade the aromatic hydrocarbons (Rockne et al. 1998). Rockne and Strand (2003) provided further evidence for the existence of type II marine methanotrophs, indicating the possibility of exploiting co-metabolic activity in remediation of marine ecosystems. The methanotrophic bioremediation is now considered to be a promising technology for the treatment of oil-contaminated marine environment as it is both environment friendly and provides low-cost degradation of toxic components of petroleum (Rockne and Strand 2003).

The most rapid and complete degradation of the majority of organic pollutants is generally carried out under aerobic conditions. The initial cellular attack on organic pollutants is an oxidative process mediated by activation as well as incorporation of molecular oxygen through key enzymatic reaction, particularly catalysed by oxygenases (Das and Chandran 2011). The methane monooxygenases (i.e. sMMO and pMMO) present in diverse type of obligate aerobic methanotrophs can actively participate in the degradation of various alkanes (Table 1.2) as suggested by van Beilen and Funhoff (2005).

But, the knowledge and understanding of bioremediation of petroleum hydrocarbons in polar environment by microbes is limited (Simpson et al. 1995). In extreme habitats such as in the Polar Regions, very cold and fluctuating temperature, low nutrient levels, stressful moisture and alkaline pH conditions do not favour an efficient biodegradation process (Thomassin-Lacroix et al. 2002; Rike et al. 2005).

Table 1.2 Methanotrophic methane monooxygenase (MMO) enzymes involved in the biodegradation of petroleum hydrocarbons

MMO enzymes	Methanotrophs	Petroleum contaminants	References
Soluble methane monooxygenase (sMMO)	*Methylococcus, Methylosinus, Methylocystis, Methylomonas, Methylocella*, etc.	C_1–C_8 alkanes, alkenes and cycloalkanes	McDonald et al. (2006)
Particulate methane monooxygenase (pMMO)	*Methylobacter, Methylococcus, Methylocystis,* etc.	C_1–C_5 (halogenated) alkanes and cycloalkanes	McDonald et al. (2006)

Modified from Das and Chandran (2011)

Since the process is slow, it also leaves behind residual toxic intermediates (Pelletier et al. 2004). Investigations have been carried out to evaluate the importance of biostimulation by fertilizing the soil with N and P so as to enhance the biodegradation of hydrocarbons (Whyte et al. 1999, 2002). Pure cultures of psychrophilic and psychrotolerant methanotrophs isolated and characterized as new genera are as follows: *Methylobacter psychrophilus*, *Methylosphaera hansonii*, *Methylocella palustris*, *Methylocella silvestris*, *Methylocella tundrae*, *Methylocapsa acidiphila* and *Methylomonas scandinavica* (Trotsenko and Khmelenina 2005). These isolated psychrophiles are capable of growing at freezing temperatures (0 °C) and exhibit optimum growth between 10 and 13 °C. The combination of cold adaptation and seawater requirements appears to be a frequent event, which has been observed in these Antarctic psychrophilic isolates (Bowman et al. 1997). Trotsenko and Khmelenina (2005) suggested that even after long-term storage in permafrost, some methanotrophs can oxidize the carbon substrate. The presence of six sterols, lanosterol, lanost-8(9)-en-3β-ol, 4,4-dimethylcholesta-8(14), 24-dien-3β-ol, 4,4-dimethylcholest-8(14)-en-3β-ol, 4-methylcholesta-8(14),24-dien-3β-ol and 4-methylcholest-8(14)-en-3β-ol, in the psychrophilic methanotrophic bacterium, *Methylosphaera hansonii*, indicated its capability to survive over a diverse range of temperatures (Schouten et al. 2000; Sinninghe Damste et al. 2000). However, in fact comparatively very little information are available about nature of extremophilic methanotrophs, dynamics of their diversity in contaminated sites, the genes that confer them the capability for bioremediation and their survival in the extreme environmental conditions.

1.4 Methanotrophs in Halogenated Hydrocarbon Remediation

The pollution of natural environment like groundwater and soil by halogenated hydrocarbons has become a serious ecological problem (Kikuchi et al. 2002; Takeuch et al. 2005). Low-molecular-weight halogenated hydrocarbons are susceptible to degradation by anaerobic and aerobic bacteria Hanson et al. (1990). Methanotrophic bacterium *Methylosinus trichosporium* 0B3b degrades TCE more rapidly than other bacteria and a correlation between the synthesis of sMMO and TCE biodegradation was confirmed. Chlorinated ethenes are synthetic compounds with no recognized natural sources and are commonly applied in diverse business practices including degreasing operations, dry cleaning, dying, textile production, etc. (Bakke et al. 2007; van Hylckama Vlieg and Janssen 2001). The reductive anaerobic bioremediation of chlorinated hydrocarbons, for example tetrachloroethylene to ethene through TCE, dichloroethylene (DCE), and vinyl chloride (VC) as intermediates, has been known for some time (Maymo-Gatell et al. 1999). However, in situ application of anaerobic biodechlorination has been imperfect as this process does not result in complete dechlorination in the presence of sulphate due to metabolic competition with the sulphate-reducing bacteria for hydrogen (Daugulis and McCracken 2003; Singh et al. 2008). Thus, incomplete dechlorination leads to accumulation of TCE, *cis*-dichloroethylene (*c*-DCE), *trans*-dichloroethylene (*t*-DCE) and VC (Maymo-Gatell et al. 1999).

There have been dearth of information on aerobic bacterial strains that can consume halogenated hydrocarbons such as chlorinated ethenes as growth substrates (Verce et al. 2000; Coleman et al. 2002) or co-metabolize these compounds (Futamata et al. 2001). Population density of methanotrophic bacteria in rhizosphere soils of vascular plants contaminated with a mixture of chemicals, including TCE showed significantly higher number of methanotrophic bacteria (Brigmon et al. 1999).

Methanotrophic bacteria are one of those groups of microbes capable of degrading these hazardous compounds via co-oxidation. Due to their omnipresence in diverse environment, these bacteria have been widely applied for cleaning the sites contaminated with chlorinated ethenes (Hanson and Hanson 1996; Semrau et al. 2010). Due to capability of methanotrophs to degrade a wide variety of potential pollutants including halogenated hydrocarbons has prompted the workers to study their potential applications in bioremediation (Lontoh et al. 2000; Nikiema et al. 2005; Lee et al. 2006). Application of high levels of biostimulating substances could cause other problems with environmental pollutants due to their interaction with organic compounds. In contrast, methanotrophs induce the MMO involved in TCE degradation only in the presence of CH_4 (Takeuchi et al. 2005; Shukla et al. 2009). Since the CH_4 is one of the natural end products of anaerobic microbial processes it should not cause environmental problems at the levels of bioremediation of organic contaminants. Due to all the required considerations and precautions, in situ-stimulated bioremediation by augmenting the methanotrophic populations is under way in many laboratories (Pfiffner et al. 1997; Iwamoto et al. 2000).

An increase in the population of indigenous methanotrophs due to addition of nutrients and natural gas to a sand column demonstrated that the TCE was degraded to carbon dioxide (Wilson and Wilson 1985). The abundance of methanotrophs in the TCE-contaminated aquifers in a natural gas field implied that the coarse sand stratum plays an important role for in situ bioremediation (Takeuchi et al. 2001). Expanding this idea of in situ bioremediation, additional considerations for selection of microbes as well as suitable habitat are the primary requirement for successful bioremediation. The diversity of the methanotrophic community involved in degradation of TCE from non-contaminated environment provides an indication of the in situ bioremediation potential of natural soil environments (Newby et al. 2004; Erwin et al. 2005).

1.5 Methanotrophs in Lindane Remediation

Lindane-contaminated soils cause potentially serious problems to surface and ground water quality, especially when its concentration is high due to unwarranted spills or discharges (Singh 2008). It is still considered to be a serious threat to the environment due to its persistent nature in environment and its potential to bioaccumulate in the food chain. Though the γ-HCH is biodegradable, higher concentrations are inhibitory to the degradation potential of applied microbes (Abhilash and Singh 2008). A significant increase in the population densities of methanotrophs in the soil contaminated by γ-HCH indicated the survival capacity of these microbes

against the insecticide lindane (Rubinos et al. 2007). A possible way to improve the bioremediation efficiency is possible through application of native methanotrophic bacteria adapted to the contaminated site. Slow-release bioaugmentation approach, using encapsulated *Sphingomonas* spp. cells, for the biodegradation of lindane in laboratory condition has also been used (Bhatt et al. 2007). Therefore, bioremediation based on methanotrophic bacteria might be an emerging tool and has been receiving more attention as an eco-friendly and efficient means of lindane remediation (Mertens et al. 2005). There is need for further improvement in the technology in order to achieve a reliable bioaugmentation technology for bioremediation of lindane-contaminated sites. Emphasis should be laid on the enhanced effort to screen for more indigenous methanotrophic population and appropriate inoculation practice to optimize the technology.

1.6 Plant–Methanotrophs Associations in Bioremediation

A plant–microbe association has been recognized as an important relationship for benefit of both the partners as well as for sustainable ecosystem functioning (Singh 2015c). A viable methanotroph–plant association in the soil environment could be imperative to create a favourable condition for better performance of methanotrophs with respect to bioremediation as well as methane removal from the environment. Such environment supporting the plant–methanotrophs association can greatly benefit the plants as growth-promoting agents. However, a mutualistic association between transgenic methanotrophs and plants augmenting the bioremediation of contaminated sites is still conjecturable (Pandey et al. 2014). The information related to plant–methanotrophs associations in soils and their co-operative role in methane consumption and bioremediation of toxic substances from various polluted soils are almost lacking. Therefore, there is an urgent need to investigate and exploit plant–methanotrophs interaction services for mutual benefit of both plant and methanotrophs that may enhance and stabilize the environmental sustainability.

1.7 Can Extremophilic Methanotrophs (*Verrucomicrobia*) Be Used for Bioremediation?

Due to various sophisticated and advanced molecular tools, the methanotrophs well adapted to extreme environmental conditions are not a remote possibility (Tiwari et al. 2015). Various extremophilic species of aerobic methanotrophic bacteria including psychrophiles, thermophiles, acidophiles, halophiles, alkaliphiles, etc. have been reported and isolated in pure culture from the environment (Dunfield 2009). These extremophilic methanotrophs have been placed in a separate phylogenetic group known as *Verrucomicrobia*. Given their ability to survive in extremophilic conditions, it is possible that these extremophilic methanotrophic strains can be exploited as potential bioagents to degrade various noxious pollutants under a wide range of environmental conditions.

As acidophilic methanotrophs show interesting possibilities for their application in bioremediation of pollutants, the findings on thermoacidophilic methanotrophs within the *Verrucomicrobia* phylum are presumably going to open new avenues for potential use of these extremophilic methanotrophs as new bioremediation tools. There have been no reports on thermoacidophilic methanotrophs which degrade various pollutants mediated by MMOs. Recently, it has been reported that CO_2 is assimilated by extremophilic methanotrophs similar to facultative methanotrophs; the use of C sources instead of CH_4 may increase the usefulness of these bioagents for bioremediation. However, it should be kept in mind that CH_4 utilization by these microbes is the primary source of energy (Khadem et al. 2011). It is now well known that many of these extremophilic methanotrophic strains, predominantly species within the *Methylocella* genera, are capable to synthesize the sMMO, but none are reported to have the potential to degrade the pollutants (Tiwari et al. 2015). Hence, an extensive effort is required to proceed cautiously on this new emerging area of bioremediation by methanotrophs, particularly these unique extremophilic methanotrophs. There is still need to isolate and identify the methanotrophs from extreme habitats and explore their potential role in degradation of hazardous chemicals. Further researches on extremophilic methanotrophs isolated from extreme environment should focus on isolation of strains with cellular tolerance to different concentrations of pollutants. The natural diversity of extremophilic methanotrophs using the functional genes involved in the remediation of complex needs to be tested against persistent noxious pollutants.

1.8 Conclusions and Future Research Opinions

Methanotrophic bacteria have a ubiquitous distribution in the environment and the use of natural gas or methane with other nutrients is used to stimulate their bioremediation potential through MMO enzymes. The pMMO-expressing methanotrophs may be preferred over sMMO-expressing methanotrophs over a broad range of pollutant concentrations as those microorganisms are better able to bind and turn over methane in the presence of competing compounds, and thus increase cell numbers and generate reducing equivalents, both of which enhance pollutant degradation. The methanotrophic MMOs potential to degrade diverse toxic pollutants have led to greater commercial application of this unique microbe. Bioaugmentation of contaminated sites with microbial cells continues to be a source of argument within the community of environmental microbiologists. Therefore, there is need to develop a reliable bioaugmentation technology for the biodegradation of toxicants (Shukla et al. 2010) by surveying the most suitable methanotrophic strains and a suitable inoculation procedure for their introduction into the environment. Under extreme adverse conditions, a number of methanotrophs have shown potential to facilitate the degradation of several toxicants.

Based on the molecular techniques, *Verrucomicrobia* (a group of extremophilic methanotrophs) has been identified in a broad range of aquatic and terrestrial habitats (Fig. 1.4). In soil system, they can cover up to 1–10 % of the total bacterial 16s RNA,

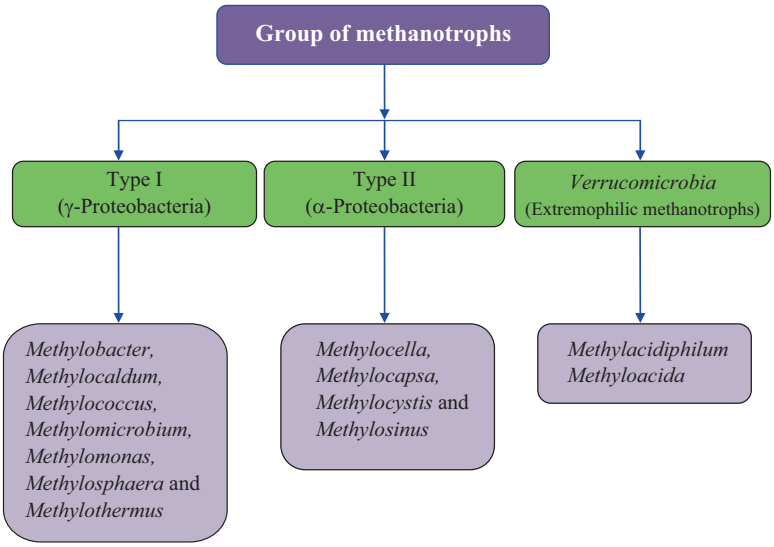


Fig. 1.4 Diverse methanotrophs genera reported from various types of ecosystems

indicating that the microbes of this group could play a very crucial ecological role (Islam et al. 2008). A unique thermoacidophilic methanotroph, i.e. *Methyloacida kamchatkensis* (Kam1) belonging to the *Verrucomicrobia* phylum has been reported from an acidic hot spring in Kamchatka, Russia. Due to its unusual phylogenetic position, apparent lack of classical methane oxidation genes and intracellular membrane systems (ICM), and the presence of polyhedral organelles, the extremophilic methanotroph *M. kamchatkensis* is a novel type of methane-oxidizing system which could be exploited to develop bioremediation machinery for extreme environmental conditions. The polyhedral organelle in Kam1 may substitute for ICM and result in a novel subcellular compartment for methane oxidation. It may be speculated that the *M. kamchatkensis*, with unique polyhedral organelles offer an efficient methanotroph for bioremediation of various pollutants. Further biochemical and genomic studies of *M. kamchatkensis* are expected to provide insight into the evolution and adaptation of the methane oxidation pathways and bioremediation mechanisms under extreme environmental habitats.

A reasonable in situ bioremediation of pollutants requires designing of unique ecological niches or microhabitats along with genetic manipulation of the organisms capable of showing better performance in bioremediation (Davidson 2005; Ward et al. 2004). Further, application of locally isolated indigenous methanotrophs (type I, II or X) which are well adapted to local climatic conditions can be a boon to bioremediation technology. Methanotrophic in situ bioremediation appears to be a viable and interesting topic of future research. Hence, a deep understanding of physiology and genetics of methanotrophs in detail may prove to be a very useful tool for improved bioremediation.

Acknowledgement Financial support from UGC-BSR Project, New Delhi, Govt. of India and BBAU, Lucknow is gratefully acknowledged.

References

Abhilash PC, Singh N (2008) Influence of the application of sugarcane bagasse on lindane (γ-HCH) mobility through soil column: implication for biotreatment. Bioresour Technol 99:8961–8966

Aislabie J, Balks MR, Foght M, Waterhouse EJ (2004) Hydrocarbon spills on Antarctic soils: effects and management. Environ Sci Technol 38:1265–1274

Bakke B, Stewart PA, Waters MA (2007) Uses of and exposure to trichloroethylene in US industry: a systematic literature review. J Occup Environ Hyg 4:375–390

Bhatt P, Kumar MS, Chakrabarti T (2007) Assessment of bioremediation possibilities of technical grade hexachlorocyclohexane (tech-HCH) contaminated soils. J Hazard Mate 143:349–353

Boden R, Murrell JC (2011) Response to mercury (II) in *Methylococcus capsulatus* (Bath). FEMS Microbiol Lett 324:106–110

Bolt HM (2005) Vinyl chloride—a classical industrial toxicant of new interest. Crit Rev Toxicol 35:307–323

Bowman JP, McCammon SA, Skerratt JH (1997) *Methylosphaera hansonii* gen. nov., sp. nov., a psychrophilic, group I methanotroph from Antarctic, marine salinity, meromictic lakes. Microbiology 143:1451–1459

Brigmon RL, Anderson TA, Fliermans CB (1999) Methanotrophic bacteria in the rhizosphere of trichloroethylene-degrading plants. Inter J Phytorem 1:241–253

Brooijmans RJW, Pastink MI, Siezen RJ (2009) Hydrocarbon-degrading bacteria: the oil-spill clean-up crew. Microb Biotechnol 2:587–594

Cervantes C, Campos-Garcıa J, Devars S, Gutierrez-Corona F, Loza-Tavera H, Torres-Guzman JC, Moreno-Sanchez R (2001) Interactions of chromium with microorganisms and plants. FEMS Microbiol Lett 25:335–347

Choi DW, Do YS, Zea CJ, McEllistrem MT, Lee SW et al (2006) Spectral and thermodynamic properties of Ag(I), Au(III), Cd(II), Co(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(IV), and Zn(II) binding by methanobactin from *Methylosinus trichosporium* OB3b. J Inorg Biochem 100:2150–2161

Coleman NV, Mattes TE, Gossett JM, Spain JC (2002) Phylogenetic and kinetic diversity of aerobic vinyl chloride-assimilating bacteria from contaminated sites. Appl Environ Microbiol 68:6162–6171

Das N, Chandran P (2011) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 11:1–13

Das K, Mukherjee AK (2007) Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresour Technol 98:1339–1345

Daugulis AJ, McCracken CM (2003) Microbial degradation of high and low molecular weight polyaromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of *Sphingomonas* sp. Biotechnol Lett 25:1441–1444

Davidson J (2005) Risk mitigation of genetically modified bacteria and plants designed for bioremediation. J Ind Microbiol Biotechnol 32:639–650

Dedysh SN, Liesack W, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Bares AM et al (2000) *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. Int J Syst Evol Microbiol 50:955–969

DeMarco P, Pacheco CC, Figueiredo AR, Moradas-Ferreira P (2004) Novel pollutant-resistant methylotrophic bacteria for use in bioremediation. FEMS Microbiol Lett 234:75–80

Erwin DP, Erickson IK, Delwiche ME, Colwell FS, Strap JL, Ronald LC (2005) Diversity of oxygenase genes from methane- and ammonia-oxidizing bacteria in an Eastern Snake River Plain Aquifer. Appl Environ Microbiol 71:2016–2025

Ferris FG, Konhauser KO, Lyven B, Pedersen K (1999) Accumulation of metals by bacteriogenic iron oxides in a subterranean environment. Geomicrobiol J 16:181–192

Futamata H, Harayama S, Watanabe K (2001) Diversity in kinetics of trichloroethylene-degrading activities exhibited by phenol-degrading bacteria. Appl Microbiol Biotechnol 55:248–253

Hanson RS, Hanson TS (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471

Hanson RS, Tsien HC, Tsuji K, Brusseau GA, Wackett LP (1990) Biodegradation of low molecular-weight halogenated hydrocarbons by methanotrophic bacteria. FEMS Microbiol Lett 87:273–278

Hasin AA, Gurman SJ, Murphy LM, Perry A, Smith TJ, Gardiner PE (2010) Remediation of chromium (VI) by a methane-oxidizing bacterium. Environ Sci Technol 44:400–405

Hazen TC, Chakraborty R, Fleming JM, Gregory IR, Bowman JP, Jimenez L, Zhang D, Pfiffner SM, Brockman FJ, Sayler GS (2009) Use of gene probes to assess the impact and effectiveness of aerobic *in situ* bioremediation of TCE. Arch Microbiol 191:221–232

Henry SM, Grbic-Galic D (1994) Biodegradation of trichloroethylene in methanotrophic systems and implications for process applications. In: Chaudhry GR (ed) Biological degradation and bioremediation of toxic chemicals. Dioscorides Press, Portland, pp 314–344

Im J, Semrau JD (2001) Pollutant degradation by a *Methylocystis* strain SB2 grown on ethanol: bioremediation via facultative methanotrophy. FEMS Microbiol Lett 318:137–142

Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK (2008) Methane oxidation at 55 °C and pH 2 by a thermoacidophilic bacteria belonging to the *Verrucomicrobia* phylum. Proc Nat Acad Sci U S A 105:300–304

Iwamoto T, Tani K, Nakamura K, Suzuki Y, Kitagawa M, Eguchi M, Nasu M (2000) Monitoring impact of in situ biostimulation treatment on groundwater bacterial community by DGGE. FEMS Microbiol Ecol 32:129–141

Jenkins MB, Chen J-H, Kadner DJ, Lion LW (1994) Methanotrophic bacteria and facilitated transport of pollutants in aquifer material. Appl Environ Microbiol 60:3491–3498

Jiang H, Chen Y, Jiang PX, Zhang C, Smith TJ, Murrell JC, Xing XH (2010) Methanotrophs: multifunctional bacteria with promising applications in environmental bioengineering. Biochem Eng J 49:277–288

Kikuchi T, Iwasaki K, Nishihara H, Takamura Y, Yagi O (2002) Quantitative and rapid detection of the trichloroethylene-degrading bacterium *Methylocystis* sp. M in groundwater by real-time PCR. Appl Microbiol Biotechnol 59:731–736

Kim HJ, Graham DW, DiSpirito AA, Alterman MA, Galeva N, Larive CK, Asunskis D, Sherwood PMA (2004) Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. Science 305:1612–1615

Knapp CW, Fowle DA, Kulczycki E, Roberts JA, Graham DW (2007) Methane monooxygenase gene expression mediated by methanobactin in the presence of mineral copper sources. Proc Nat Acad Sci U S A 104:12040–12045

Lee SW, Keeney DR, Lim DH, Dispirito AA, Semrau JD (2006) Mixed pollutant degradation by *Methylosinus trichosporium* OB3b expressing either soluble or particulate methane monooxygenase: can the tortoise beat the hare? Appl Environ Microbiol 72:7503–7509

Lontoh S, Semrau JD (1998) Methane and trichloroethylene degradation by *Methylosinus trichosporium* OB3b expressing particulate methane monooxygenase. Appl Environ Microbiol 64:1106–1114

Lontoh S, Zahn JA, DiSpirito AA, Semrau JD (2000) Identification of intermediates of in vivo trichloroethylene oxidation by the membrane-associated methane monooxygenase. FEMS Microbiol Lett 186:109–113

Maymo-Gatell X, Anguish T, Zinder SH (1999) Reductive dechlorination of chlorinated ethenes and 1, 2-dichloroethane by *Dehalococcoides ethenogenes* 195. Appl Environ Microbiol 65:3108–3113

McCue T, Hoxworth S, Randall AA (2002) Degradation of halogenated aliphatic compounds utilizing sequential anaerobic/aerobic treatments. Water Sci Technol 47:79–84

McDonald IR, Miguez CB, Rogge G, Bourque D, Wendlandt KD, Groleau D, Murrell JC (2006) Diversity of soluble methane monooxygenase containing methanotrophs isolated from polluted environments. FEMS Microbiol Lett 255:225–232

Mertens B, Boon N, Verstraete W (2005) Stereospecific effect of hexachlorocyclohexane on activity and structure of methanotrophic communities. Environ Microbiol 7:660–669

Newby DT, Reed DW, Petzke LM, Igoe AL, Delwiche ME, Roberto FF, McKinley JP, Whiticar MJ, Colwell FS (2004) Diversity of methanotroph communities in a basalt aquifer. FEMS Microbiol Ecol 48:333–344

Nikiema J, Bibeau L, Lavoie J, Brzezinski R, Vigneux J, Heitz M (2005) Biofiltration of methane: an experimental study. Chem Eng J 113:111–117

Oldenhuis R, Oedzes JY, Van Der Waarde JJ, Janssen DB (1991) Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichosporium* OB3b and toxicity of trichloroethylene. Appl Environ Microbiol 57:7–14

Øverland M, Tauson AH, Shearer K, Skrede A (2010) Evaluation of methane-utilizing bacteria products as feed ingredients for monogastric animals. Arch Anim Nutr 64:171–189

Pandey VC, Singh JS, Singh DP, Singh RP (2014) Methanotrophs: promising bacteria for environmental remediation. Int J Environ Sci Technol 11(1):241–250

Pelletier E, Delille D, Delille B (2004) Effectiveness of crude oil bioremediation in sub-Antarctic, intertidal sediments; chemistry and toxicity of oiled residues. Mar Environ Res 57:311–327

Pfiffner SM, Palumbo AV, Phelps TJ, Hazen TC (1997) Effects of nutrient dosing on subsurface methanotrophic populations and trichloroethylene degradation. J Ind Microbiol Biotechnol 18:204–212

Rike AG, Haugen KB, Engene B (2005) In situ bioremediation of hydrocarbons in Arctic soil at sub-zero temperature-field monitoring and theoretical simulation of the microbial activation temperature at a Spitsberg contaminated site. Cold Reg Sci Technol 41:189–209

Rockne KJ, Strand SE (2003) Amplification of marine methanotrophic enrichment DNA with 16S rDNA PCR primers for type II alpha proteobacteria methanotrophs. J Environ Sci Health A Tox Hazard Subst Environ Eng 38:1877–1887

Rockne KJ, Stensel HD, Herwig RP, Strand SE (1998) PAH degradation and bioaugmentation by a marine methanotrophic enrichment. Bioremed J 1:209–222

Rubinos DA, Villasuso R, Muniategui S, Barral MT, Diaz-Fierros F (2007) Using the land farming technique to remediate soils contaminated with hexachlorocyclohexane isomers. Water Air Soil Pollut 181:385–399

Scott CS, Chiu WA (2006) Trichloroethylene cancer epidemiology: a consideration of select issues. Environ Health Perspect 114:1471–1478

Semrau JD, DiSpirito AA, Yoon S (2010) Methanotrophs and copper. FEMS Microbiol Rev 34:496–531

Shigematsu T, Hanada S, Eguchi M, Kamagata Y, Kanagawa T, Kurane R (1999) Soluble methane monooxygenase gene clusters from trichloroethylene-degrading *Methylomonas* sp. strains and detection of methanotrophs during *in situ* bioremediation. Appl Environ Microbiol 65:5198–5206

Shukla AK, Vishwakarma P, Upadhyay SN, Tripathi AK, Prasana HC, Dubey SK (2009) Biodegradation of trichloroethylene (TCE) by methanotrophic community. Bioresour Technol 100:2469–2474

Shukla KP, Singh NK, Sharma S (2010) Bioremediation: developments, current practices and perspectives. Gen Eng Biotechnol J 10:1–20

Simpson RD, Smith SDA, Pople AR (1995) The effects of a spillage of diesel fuel on a rocky shore in the sub-Antarctic region (Macquarie Island). Mar Pollut Bull 31:367–371

Singh DK (2008) Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Ind J Microbiol 48:35–40

Singh JS (2011) Methanotrophs: the potential biological sink to mitigate the global methane load. Curr Sci 100(1):29–30
Singh JS (2013a) Anticipated effects of climate change on methanotrophic methane oxidation. Clim Change Environ Sustain 1(1):20–24
Singh JS (2013b) Plant growth promoting rhizobacteria: potential microbes for sustainable agriculture. Resonance 18(3):275–281
Singh JS (2014) Cyanobacteria: a vital bio-agent in eco-restoration of degraded lands and sustainable agriculture. Clim Change Environ Sustain 2:133–137
Singh JS (2015a) Microbes: the chief ecological engineers in reinstating equilibrium in degraded ecosystems. Agric Ecosyst Environ 203:80–82
Singh JS (2015b) Biodiversity: current perspectives. Clim Change Environ Sustain 2:133–137
Singh JS (2015c) Plant-microbe interactions: a viable tool for agricultural sustainability. Appl Soil Ecol 92:45–46
Singh JS, Gupta VK (2016) Degraded land restoration in reinstating CH_4 sink. Front Microbiol 7(923):1–5
Singh JS, Pandey VC (2013) Fly ash application in nutrient poor agriculture soils: impact on methanotrophs population dynamics and paddy yields. Ecotoxicol Environ Saf 89:43–51
Singh JS, Singh DP (2012) Reforestation: a potential approach to mitigate the excess CH_4 build-up. Ecol Manag Restor 13(3):245–248
Singh JS, Singh DP (2013a) Impact of anthropogenic disturbances on methanotrophs abundance in dry tropical forest ecosystems, India. Expert Opin Environ Biol 2:1–3
Singh JS, Singh DP (2013b) Plant Growth Promoting Rhizobacteria (PGPR): microbes in sustainable agriculture. In: Malik A, Grohmann E, Alves M (eds) Management of microbial resources in the environment. Springer, Netherlands, pp 307–319
Singh JS, Strong PJ (2016) Biologically derived fertilizer: a multifaceted bio-tool in methane mitigation. Ecotoxicol Environ Saf 124:267–276
Singh S, Mulchandani A, Chen W (2008) Highly selective and rapid arsenic removal by metabolically engineered *Escherichia coli* cells expressing *Fucus vesiculosus* metallothionein. Appl Environ Microbiol 74:2924–2927
Singh JS, Pandey VC, Singh DP, Singh RP (2010) Influence of pyrite and farmyard manure on population dynamics of soil methanotroph and rice yield in saline rain-fed paddy field. Agri Eco Environ 139:74–79
Singh JS, Abhilash PC, Singh HB, Singh RP, Singh DP (2011a) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480:1–9
Singh JS, Pandey VC, Singh DP (2011b) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353
Singh JS, Pandey VC, Singh DP (2011c) Coal fly ash and farmyard manure amendments in dryland paddy agriculture field: effect on N-dynamics and paddy productivity. Appl Soil Ecol 47:133–140
Singh JS, Singh DP, Dixit S (2011d) Cyanobacteria: an agent of heavy metal removal. In: Maheshwari DK, Dubey RC (eds) Bioremediation of pollutants. IK International Publisher, New Delhi, pp 223–243
Singh JS, Kumar A, Rai AN, Singh DP (2016) Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front Microbiol 7(529):1–19
Sinninghe Damste JS, Schouten S, Bowman JP, Rijpstra WIC (2000) Sterols in a psychrophilic methanotroph, *Methylosphaera hansonii*. FEMS Microbiol Lett 186:193–195
Stein LY, Yoon S, Semrau JD, DiSpirito AA, Crombie A, Murrell JC, Vuilleumier S, Kalyuzhnaya MJ, Op den Camp HJM, Bringel F, Bruce D, Cheng JF, Copeland A, Goodwin L, Han S, Hauser H, Jetten MSM, Lajus A, Land ML, Lapidus A, Lucas S, Medigue C, Pitluck S, Woyke T, Zeytun A, Klotz MG (2010) Genome sequence of the obligate methanotroph *Methylosinus trichosporium* strain OB3b. J Bacteriol 192:6497–6498

Sullivan JP, Dickinson D, Chase HA (1998) Methanotrophs, *Methylosinus trichosporium* OB3b, sMMO, and their application to bioremediation. Crit Rev Microbiol 124:335–373

Takeuchi M, Nanba K, Iwamoto H, Nirei H, Kusuda T, Kazaoka O, Furuya K (2001) Distribution of methanotrophs in trichloroethylene-contaminated aquifers in a natural gas field. Geomicrobiol J 18:387–399

Takeuchi M, Nanba K, Yoshida M, Nirei H, Furuya K (2004) Natural groundwater of a gas field utilizable for a bioremediation of trichloroethylene-contamination. Environ Geol 45:891–898

Takeuchi M, Nanba K, Iwamoto H, Nirei H, Kusuda T, Kazaoka O, Owaki M, Furuya K (2005) *In situ* bioremediation of a cis-dichloroethylene-contaminated aquifer utilizing methane-rich groundwater from an uncontaminated aquifer. Water Res 39:2438–2444

Thomassin-Lacroix EJM, Eriksson M, Reimer KJ, Mohn WW (2002) Biostimulation and bioaugmentation for on-site treatment of weathered diesel fuel in Artic soil. Appl Microbiol Biotechnol 59:551–556

Throne-Holst M, Wentzel A, Ellingsen TE, Kotlar HK, Zotchev SB (2007) Identification of novel genes involved in long-chain n-alkane degradation by *Acinetobacter* sp. strain DSM 17874. Appl Environ Microbiol 73:3327–3332

Tiwari S, Singh JS, Singh DP (2015) Methanotrophs and CH_4 sink: effect of human activity and ecological perturbations. Clim Change Environ Sustain 3(1):35–50

Travis BJ, Rosenberg ND (1997) Modeling in situ bioremediation of TCE at Savannah River: effects of product toxicity and microbial degradation. Environ Sci Technol 31:3093–3102

Trotsenko YA, Khmelenina VN (2005) Aerobic methanotrophic bacteria of cold ecosystems. FEMS Microbiol Ecol 53:15–26

Trotsenko YA, Murrell JC (2008) Metabolic aspects of aerobic obligate methanotrophy. Adv Appl Microbiol 63:183–229

Van Beilen JB, Funhoff EG (2005) Expanding the alkane oxygenase toolbox: new enzymes and applications. Curr Opin Biotechnol 16:308–314

van Hylckama Vlieg JET, Janssen DB (2001) Formation and detoxification of reactive intermediates in the metabolism of chlorinated ethenes. J Biotechnol 85:81–102

Verce MF, Ulrich RL, Freedman DL (2000) Characterization of an isolate that uses vinyl chloride as a growth substrate under aerobic conditions. Appl Environ Microbiol 66:3535–3542

Ward N, Larsen O, Sakwa J, Bruseth L, Khouri H, Durkin AS et al (2004) Genomic insights into methanotrophy: the complete genome sequence of *Methylococcus capsulatus* (Bath). PLoS Biol 2:1616–1628

Whyte LG, Bourbonniere L, Bellerose C, Greer CW (1999) Bioremediation assessment of hydrocarbon-contaminated soils from the high Arctic. Bioremed J 3:69–79

Whyte LG, Schultz A, Van Beilen JB, Luz AP, Pellizari V, Labbe D, Greer CW (2002) Prevalence of alkane monooxygenase genes in Arctic and Antarctic hydrocarbon-contaminated and pristine soils. FEMS Microbiol Ecol 41:141–150

Wilson JT, Wilson BH (1985) Biotransformation of trichloroethylene in soil. Appl Environ Microbiol 49:242–243

Yakimov MM, Timmis KN, Golyshin PN (2007) Obligate oil-degrading marine bacteria. Curr Opin Biotechnol 18:257–266

Yoon S (2010) Towards practical application of methanotrophic metabolism in chlorinated hydrocarbon degradation, greenhouse gas removal, and immobilization of heavy metals. Ph.D. Dissertation, University of Michigan, 159 p; AAT 3429318

Zayed AM, Terry N (2003) Chromium in the environment: factors affecting biological remediation. Plant and Soil 249:139–156

Chapter 2
Methanotrophs: Methane Mitigation, Denitrification and Bioremediation

Peter James Strong, Obulisamy Parthiba Karthikeyan, Jing Zhu, William Clarke, and Weixiang Wu

Abstract Methanotrophs are bacteria capable of using methane as a carbon source. They can lower atmospheric methane emissions, remove N in environmental and wastewater treatment systems and even transform organic pollutants in soils. Methanotrophic methane mitigation technologies have been demonstrated beyond the laboratories as adaptable field-scale systems that may be engineered to meet site-specific climatic variations and ensure minimal atmospheric methane emission. In agricultural sediments and soils, methanotrophs sequester methane but are affected by fertiliser applications, while in wastewater treatment systems they can lower the costs associated with N removal. Finally, the methanotrophs are particularly appealing as bioremediation agents in methane-containing environments, as their primary enzymes have a broad substrate range that can transform various hydrocarbons, including aromatic compounds and halogenated aliphatics. These diverse bacteria are an important global methane sink and this importance is set to increase as anthropogenic emissions increase over the coming decades.

Keywords Denitrification • Landfill • Methanotrophs • Methane • VAM

P.J. Strong (✉) • W. Clarke
Centre for Solid Waste Bioprocessing, School of Civil Engineering, School of Chemical Engineering, The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia
e-mail: pjstrong@gmail.com

O.P. Karthikeyan
Sino-Forest Applied Research Centre for Pearl River Delta Environment, Hong Kong Baptist University, Hong Kong, China

College of Marine and Environmental Sciences, James Cook University, Townsville, QLD, Australia

J. Zhu • W. Wu
Institute of Environmental Science and Technology, Zhejiang University, Hangzhou, China

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_2

2.1 Introduction

Methane is a potent long-lived highly radiative gas responsible for up to 20 % of the current warming induced by greenhouse gas emissions (Kirschke et al. 2013). Global annual methane emissions are estimated at approximately 550 Tg CH_4. $year^{-1}$, and 60–70 % of this originates from biogenic sources and the rest from non-biogenic sources (IPCC 2013; Kirschke et al. 2013). Biogenic methane emissions are regulated by syntrophic microbial communities, which vary widely according to environmental factors such as temperature: moisture: salinity: pH: redox conditions: and available sulphate, nitrate and organic matter. Non-biogenic methane emission sources include geological settings, waste treatment facilities, fossil fuel industries and biomass burning, while biogenic sources include lakes, wetlands, rice cultivation, forests, livestock farming, oceans, wild animals, termites and permafrost (Kirschke et al. 2013; IPCC 2013; Karthikeyan et al. 2015; Strong et al. 2015).

Among the different microbes, methane oxidisers (primarily methanotrophs) and sulphate-reducing bacteria (SRBs) are the key microbial groups that degrade methane. Sulphate-reducing bacteria reduce sulphate into sulphide using methane as an electron donor. The SRBs are reported to be syntrophically associated with anaerobic methane-oxidising bacteria/archaea, but none of these anaerobic microbes have been isolated and the syntrophic mechanisms are still unclear (Knittel and Boetius 2009). Methanotrophs are capable of using methane as a carbon source. They are ubiquitous in nature, can be aerobic or anaerobic and serve as a global sink for methane (Hanson and Hanson 1996). Aerobic methane oxidation is well studied and many pure cultures have been isolated from various environments such as landfills, coal-bed rocks, rice fields, compost, forest soils, peat bogs, wetlands, soda lakes, thermal springs and marine sediments (Dunfield et al. 2003; Kalyuzhnaya et al. 2005; Kim et al. 2008; Hirayama et al. 2011; Lee et al. 2011; Antony et al. 2012; Saidi-Mehrabad et al. 2013). Their main enzymes for oxidising methane have broad substrate ranges (including ammonia), which allows their use in methane mitigation, bioremediation of organic pollutants and even N removal in wastewater treatment systems.

Methanotrophs were traditionally classified as Type I (gammaproteobacteria) or Type II (alphaproteobacteria), primarily according to their use of the ribulose monophosphate pathway (Type I) or serine pathways (Type II) for formaldehyde assimilation and arrangement of internal structures. They were further subdivided into a Type X group, consisting of gammaproteobacteria that had biochemical capabilities associated with Type II methanotrophs. The traditional classification scheme had its shortcomings, as the methanotrophic bacteria are more diverse and have greater biochemical capability than previously imagined. Methanotrophs are now predominantly classified according to whether they are gammaproteobacteria or alphaproteobacteria; Type X is regarded as a subdivision of Type 1 gammaproteobacteria. A recently discovered phylum that consists of thermophiles *Verrucomicrobium* (*Methylacidiphilum* and *Methylacidimicrobium* spp.) has also been added (Sharp et al. 2014; Kalyuzhnaya et al. 2015; Strong et al. 2015).

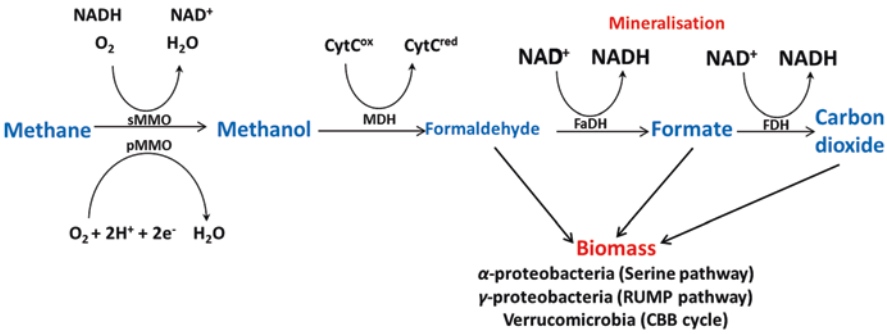


Fig. 2.1 Generalised pathways for oxidising methane to carbon dioxide, or assimilating the intermediates as biomass. *sMMO* soluble methane monooxygenase, *pMMO* particulate methane monooxygenase, *MDH* methanol dehydrogenase, *FaDH* formaldehyde dehydrogenase, *FDH* formate dehydrogenase

Methanotrophs are able to consume methane because of an enzyme called methane monooxygenase, which uses O_2 to oxidise methane to methanol. Methane monooxygenase (MMO) occurs commonly as particulate membrane-bound enzyme (pMMO) or as a soluble form (sMMO) that is synthesised in copper-deficient environments by some methanotrophs (Semrau et al. 2010). The methane monooxygenase enzymes (pMMO and sMMO) are unique functional enzymes of methanotrophs. The presence of the genes responsible (*pmoA* and *mmoX*) is particularly useful for molecular ecology studies (McDonald et al. 2008). The catalytic pathways that are initiated by the MMO enzyme are illustrated in Fig. 2.1. The pathways can split towards regenerating reducing equivalents or assimilation or into biomass. Essentially, MMO catalyses the O_2-coupled conversion of methane to methanol in methanotrophic bacteria that may be represented as follows:

$$CH_4 + 2e^- + 2H^+ + O_2 = CH_3OH + H_2O$$ (Feig and Lippard 1994; Shiemke et al. 1995).

The physiological reductant for pMMO has not been identified definitively but may involve quinones from the quinone pool reduced by a Type II NADH:quinone oxidoreductase or by methanol dehydrogenase (Culpepper and Rosenzweig 2012). The most likely physiological electron donor to pMMO is ubiquinol, but the source of electrons to reduce the resultant ubiquinone is not yet substantiated (Kalyuzhnaya et al. 2015). Artificial reductants such as duroquinol and NADH can be used to complete the oxidation (Shiemke et al. 1995). Methane monooxygenase (sMMO in particular) has a broad substrate range that includes various hydrocarbons and halogenated hydrocarbons (Jiang et al. 2010). Methane monooxygenase is also capable of oxidising ammonium, which means methanotrophs participate in the global cycling of nitrogen and methane. In natural systems, methanotrophs may play an important role in the nitrogen cycle and contribute significantly to nitrification in the rhizosphere. The relationships of methanotrophs within microbial communities are complex and can be affected by N type and availability; the complexity is compounded by their ability to fix CO_2 (Chistoserdova et al. 2005; Jiang et al. 2010; Smith and Murrell 2010) and N_2 (Pfluger et al. 2011; Singh and Strong 2015).

2.2 Methane Mitigation in Soils Associated with Agriculture, Coal Mining and Landfills

Globally, agricultural activities (including livestock farming); waste management (including landfilling); and fossil fuel retrieval, processing and delivery (including coal mining) are the three largest sources of anthropogenic methane (Hanson and Hanson 1996). Biological methane oxidation is vitally important to reduce these emissions. It is predicted that methanotrophs consume up to 40 Tg CH_4 $year^{-1}$ and sequester more than 50 % of the methane produced in soils (IPCC 2001; Reeburgh 2003; Reeburgh et al. 1993). The ability of the methanotrophs to lower methane emissions and degrade hazardous organic compounds has been reviewed (Hanson and Hanson 1996; Jiang et al. 2010; Semrau et al. 2010; Smith and Dalton 2004; Wendlandt et al. 2010). Methane oxidation rates may vary according to methane and oxygen concentrations. The following environmental variables (based on laboratory studies) regulate methane oxidation in soil:

- *Temperature.* Most methanotrophs are mesophilic and function optimally within a temperature ranging from 25 to 35 °C. Methane oxidation may cease at temperatures below 10 °C. Type I methanotrophs tend to have lower temperature optima and become more prolific under these conditions (Börjesson et al. 2004; Gebert et al. 2003).
- *Oxygen supply.* Methanotrophic bacteria are obligate aerobes that can achieve optimum methane conversion rates even at low oxygen concentrations. For biofilters, methane oxidation only commenced when oxygen levels were above 1.7 %, and maximum methane oxidation rates were achieved at approximately 9 % oxygen content (Gebert et al. 2003).
- *Nutrients.* Inorganic N (ammonium/nitrate) might stimulate or inhibit methane oxidation in soils depending on N type and its concentration, methane concentration, pH and methanotroph species present. Methanotrophic bacteria have a relatively high N demand: 0.25 mole of N is required for every mole of assimilated carbon.
- *Moisture.* Soil pore volume strongly affects this parameter, but an optimum soil moisture content is generally between 10 and 20 % w/w. Too little moisture (<5 %) significantly lowers oxidation activity due to desiccation, while too much moisture inhibits gas transfer—molecular diffusion is approximately 10,000 times slower through water than air (Cabral et al. 2007).

2.2.1 Agriculture: Rice Paddy Soils

Modern agriculture has increased in scale and intensity and production is expected to double by 2050 because of greater food, feed and energy demands (Raja 2013). Meeting this growing demand will require more land and greater crop production efficiencies. Inevitably, this will lead to increased fertiliser use, which will impact the methane flux from agricultural soils as the microbially mediated production and consumption of methane is regulated by soil physico-chemical properties and strongly

impacted by fertiliser use, crop type, irrigation and organic amendment (Zheng et al. 2010). Nitrogen fertilisers containing ammonium or nitrate are widely recognised as one of the key factors affecting methane oxidation in agricultural soils (Kravchenko et al. 2002; Seghers et al. 2003). However, reports are contradictory due to unaccounted for variability of the sample sites. Ammonium-based fertilisers have caused inhibition (Hütsch et al. 1994), stimulation (Mohanty et al. 2006) or had no effect on methane oxidation (Delgado and Mosier 1996). Ammonium can inhibit methanotrophs by outcompeting methane oxidation by MMOs, generating hydroxylamine, which prevents assimilation and energy production. Ammonium inhibition was a common assumption applied to various ecosystems, until Bodelier et al. (2000) observed ammonium-stimulated methane oxidation and methanotroph growth in rice paddy soils. Methanotrophs are significant contributors to nitrification in the rhizosphere of model microcosms associated with rice plants (Bodelier and Frenzel, 1999). Generally, the short-term use of ammonium-based fertilisers may initially prevent enzymatic methane oxidation, while the long-term use affects various populations of the soil microbial communities and can impact methane production or oxidation (Bodelier and Laanbroek 2004; Ho et al. 2014), as it may also facilitate methane production by methanogens by providing an N source (Schimel 2000).

Rice production generates a large fraction of the agriculturally generated methane, which is troubling because production as this is anticipated to increase from 600 million tonnes in 2000 to 930 million tonnes by 2030 (Kubo and Purevdorj 2004). Simple strategies, such as adopting alternate wetting and drying cycles in rice production, have delivered promising results by reducing CO_2-equivalent emissions up to 30 % (IRRI 2015). Alternatively, organic fertilisers or amendments may be incorporated into the soils. Using organic fertilisers may improve crop yields and the methane sink potential within agricultural systems, which may be further improved when combined with beneficial microbes (i.e. biofertilisers) that improve the activity of methane-oxidising bacteria such as methanotrophs. Biofertilisers may be an effective tool for agriculture that is environmentally beneficial compared to conventional inorganic fertilisers.

There are reports of the prospective role of biofertilisers with regard to methane mitigation (Singh and Strong 2015). Biofertilisers that contain aerobic photosynthetic organisms, such as *Azolla* (Yadav et al. 2014) or cyanobacteria (Mandal and Mitra 1982; Lakshmanan et al. 1994; Prasanna et al. 2002) or diazotrophs (Bhardwaj et al. 2014; Pingak et al. 2014) have lowered methane emissions from agricultural activity. Frequently, this is a result of improved dissolved oxygen availability. This has two significant effects on microbial communities. The first is that it provides oxygen that the methanotrophs require to oxidise methane, allowing for greater methane sequestration efficiencies—frequently in flooded soils poor in oxygen. Second, oxygen is toxic to the methanogens and may suppress the biological production of methane. These two outcomes have been noted to significantly lower overall methane emission normally associated with rice production. Additionally, incorporating nitrogen-fixing bacteria such as rhizobia (Rösch et al. 2002), methanotrophs (Hackl et al. 2004; Knief et al. 2003) or *Archaea* (Kemnitz et al. 2005) into a biofertiliser can increase N availability to paddy crops and lower N fertiliser requirements (Table 2.1).

Table 2.1 Amendments that improve soil and sediment fertility or decrease methane emissions.

Amendment	Beneficial role	Soil type	References
Biochars	Improve methanotroph activity	Landfill soils	Sadasivam and Reddy (2015)
Farmyard manure (pressmud) combined with pyrite	Increase methanotroph population	Salinity-disturbed paddy soils	Singh et al. (2010)
Organic amendments combined with pyrite or fly ash	Improve methanotroph activity	Salinity-disturbed paddy soils	
Organic manure combined with fly ash	Increase methanotroph population	Dry tropical nutrient-poor saline soils	Singh and Pandey (2013)
Diazotrophs *Ochrobactrum anthropi*, *Azotobacter* and *Azospirillum*	Increased O_2 content—emit less CH_4	Paddy fields	Pingak et al. (2014)
Biofertiliser *Azolla* and *Anabaena azollae*	Increased O_2 content—emit less CH_4	Flooded paddy soils	Lakshmanan et al. (1994); Prasanna et al. (2002)
Biofertiliser	Promoted rice yields and emit less CH_4	Paddy fields	Lakshmi et al. (2012)
Inoculating rice plant roots with *Azospirillum*	Increased O_2 in the rhizospheric region—emit less CH_4	Paddy fields	Sahoo et al. (2014)
Cyanobacteria: *Synechocystis*	Increased O_2 content—emit less CH_4	Paddy fields	Prasanna et al. (2002)

If agricultural output is to increase as steadily as the human population growth, sustainable and efficient tools are vitally required to mitigate methane emissions *via* natural soil microflora such as the methanotrophs, while simultaneously improving soil quality and crop yields. More research is still required to better understand the complex relationship between methane-oxidising bacteria and other soil microbes, microbially enriched organic amendments, N source, N concentration, phosphate availability, C:N ratio, to enhance the methane sink within agricultural soils.

2.2.2 Coal Mines

Coal bed or coal mine gas is a complicated gas mixture with a high methane content that is released during mining operations. Fugitive methane, emitted from coal mines around the world, represents approximately 8 % of the world's anthropogenic methane emissions (Su et al. 2005). The concentration of methane varies for different mining sites and varies locally according to coal quality and coal depth. Methane is emitted as it desorbs from coal during mining, crushing or inefficient combustion, or is actively diluted and pumped out of coal mines to prevent it reaching an explosive concentration. As with landfills, it is important to monitor, regulate and treat methane emissions from coal mines on-site. In gassy mines, the trapped methane is released as

either fugitive or as continuous emissions, with more than 70 % of the mines relying on dilution to obtain acceptable methane concentrations within the mine site (Heimann et al. 2013; Limbri et al. 2014). Here, methane is released or treated as follows:

- Ventilation air methane (VAM) is coal bed methane that is diluted with air to concentrations below 1 % (generally 0.1 to 0.7 % methane), or below the explosive limit;
- Gas drained from the coal seam or coal mine before mining at (60–95 % methane) that is generally collected for direct combustion and energy recovery; and
- Gas drained from worked, or partially worked, areas (30–95 % methane) that is either diluted or used either for energy recovery (Su et al. 2005).

There are implementation and cost barriers for biological treatment of VAM. Technically, using methanotrophic bacteria to remove methane is difficult because it is produced in large volumes with low methane concentrations (average of 0.65 %). There are issues associated with gas solubility, mass transfer, contaminant volatile organic carbon (VOCs) and particulate dust. Gas residence times represent a major hurdle for methanotroph-based biofilters. While 70 % of methane can be removed at a retention time of 15 min, longer retention times (30 min) are required for 90 % removal (Limbri et al. 2013; Sly et al. 1993). The low methane content is also problematic from a physiological perspective. Adding methanol, formate or other reducing equivalents, along with essential nutrients such as nitrogen or trace metals, is recommended to maintain cell activity (Dijk et al. 2012; Andreasen et al. 2013). Biological treatment is compounded by flow rates that fluctuate during operation because of fluctuating methane content with different coal quality and removal depths. Nonetheless, a handful of studies have assessed pilot-scale biofilters for methane removal from simulated VAM. Their results are difficult to compare directly because of differences in optimisation conditions, use of pure or mixed methanotrophs cultures, methane flow rates, gas residence times and reactor types, but high removal efficiencies of 85–98 % were achieved (Limbri et al. 2013).

Based on the operation, mine safety regulation and other methane mitigation system arrangements, the VAM flow and methane concentrations will be varied for different mine sites. It is very difficult to adapt biofilters for VAM treatment unless the methanotrophs are robust and optimised to withstand fluctuating environmental conditions. Currently, the scale of the biofilters required to treat the large volume of VAM is not economically feasible. If the carbon credits or other financial incentives are not imposed, the commercialisation of biofilter technology will struggle. However, there are potential alternatives such as the use of alternative filter packing material and the use of immobilised biocatalysts.

2.2.3 Landfills

Landfill gas (LFG) is mainly composed of methane and carbon dioxide, along with other trace gases or VOCs. Monitoring, control and treatment/prevention of LFG emissions are an integral part of landfill operations and maintenance.

The contribution of LFG emission to anthropogenic greenhouse effects has received considerable attention in recent years and much research has focused on emission control. The potential to exploit the microbial methane oxidation in biobased engineered systems was recognised by various researchers for LFG treatment (Park et al. 2002; Börjesson et al. 2004; Haubrichs and Widmann 2006; Einola et al. 2007; He et al. 2008; Park et al. 2009; Rachor et al. 2011). Generally, the landfill methane is oxidised naturally by methanotrophs in the uppermost cover layers. Various factors govern methane oxidation in landfill cover soils, *viz.* methane flux, temperature, moisture content, oxygen distribution, extracellular polymeric substances formation, ammonium content and other VOCs. Further, when the top cover soils are vegetated, plant-mediated transport mechanisms may also affect the overall methane emissions from landfills (Chanton 2005).

Biobased methane mitigation systems mimic the landfill top soil cover systems with controlled environmental conditions that support methanotrophs. There are four types of biobased methane mitigation systems: biocovers, biowindows, biofilters and biotarps (Fig. 2.2). These are considered promising and cost-effective

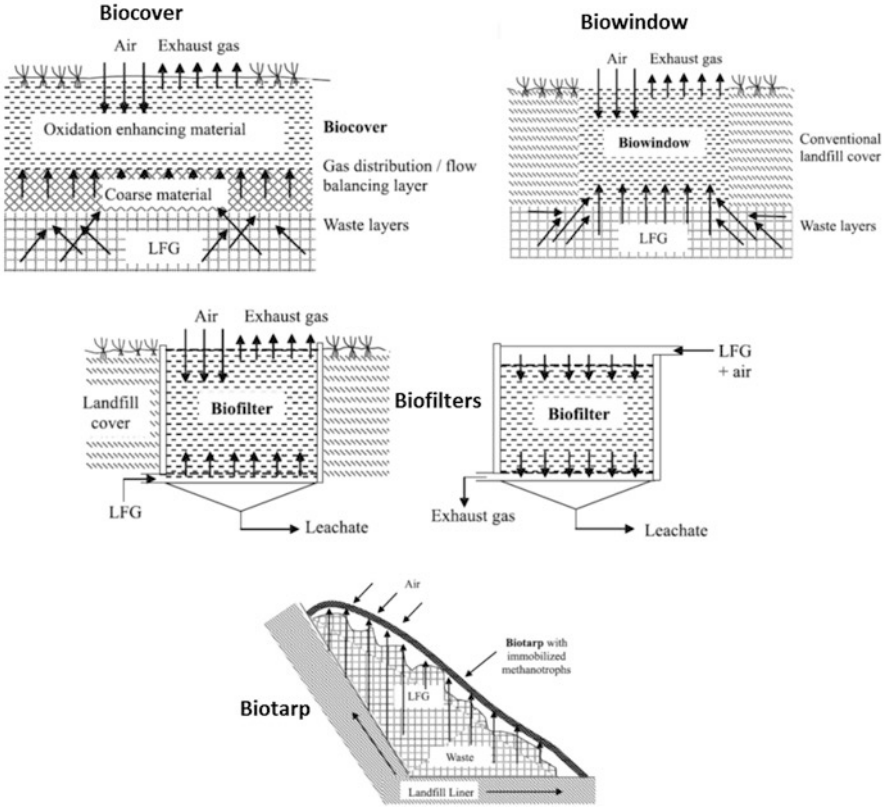


Fig. 2.2 Biobased methane mitigation systems mimic the landfill top soil cover systems (Huber-Humer et al. 2008) (This requires copyright permission)

systems that can provide methane mitigation for high or low levels of methane under prolonged conditions, i.e. during landfill operation/post-closure periods. Biobased systems can be readily configured to meet local conditions (topography and climatic conditions) and exploit naturally available materials.

2.2.3.1 Biocover

The first prototype biocover system was proposed by Humer and Lechner (1999). It consisted of a layer of coarse gravel material to provide high gas permeability and a matured well-structured compost material to support methanotroph growth. Generally, biocovers offer the advantage of full landfill coverage, where the methane flux burden is spread over a large surface area and risk of LFG emission is minimised.

2.2.3.2 Biowindow

These are similar to biocovers, but the difference is that they target relatively specific regions of landfill where point source emissions are observed. Biowindows are useful when covering the entire site is neither warranted nor economically feasible, and no gas collection system is available. Biowindow systems are generally arranged in discrete integrated structures in the top cover where LFG passively migrates through due to its increased permeability.

2.2.3.3 Biofilter

Biofilters are engineered, self-contained, fixed bed systems, packed with materials that can support/sustain methanotroph growth. In contrast to biocovers, biofilters require either an active or passive gas collection system to feed through it and is suitable when active landfill extraction and subsequent energy recovery or flaring is no longer viable or not available. They require skilled operators and are more expensive than passively vented, robust open bed applications, but they have a small footprint and high gas removal capacity (Jiang et al. 2010).

2.2.3.4 Biotarp

This is generally applied during the initial stages of landfilling to avoid early LFG emissions. It is similar to a daily cover and must be managed on a daily basis. It must be moist enough to support microbial growth but light enough to roll or fold. Its major advantage over other biobased systems is that the support matrix is inert and not subject to biochemical degradation.

The most commonly used biological solid substrates in biobased designs are mature compost, degraded or mechanically pre-treated municipal solid waste, wood chips and sludge. These biogenic materials naturally harbour methanotrophs and

are often locally available. Inorganic porous materials like gravel, clay pellets, glass beads, sands and soil are used as bulking agents in different layers. Substrate selection is important to ensure optimum conditions for microbial growth and efficient routing of LFG in biobased systems to support effective mitigation. Artificially designed and engineered media can also favour biobased systems, as they are homogeneous and have consistent physical and biochemical properties. However, in the construction of methane oxidation systems covering the large tracts of the landfill surface, huge amounts of suitable substrates are needed, and availability or costs incurred frequently limit application.

Methanotrophic methane mitigation technologies have been demonstrated as adaptable field-scale systems that may be engineered to meet site-specific climatic variations and ensure minimal atmospheric methane emission (Dever et al. 2007, 2011; Huber-Humer et al. 2008). Methane oxidation efficiencies as high as 100 % have been reported for field-scale applications (Nikiema et al. 2007; Gebert et al. 2009). Dever et al. (2011) conducted a field-scale trial at a landfill site (Sydney, Australia) investigating passive drainage and biofiltration of landfill gas as a means of managing landfill gas emissions from low to moderate gas generation landfill sites. Passively aerated biofilters operating in a temperate climate achieved maximum methane oxidation efficiencies greater than 90 % and average oxidation efficiencies greater than 50 % over 4 years of operation. Although temperature and moisture within the biofilter were affected by local climatic conditions, their effect on biofilter performance was overshadowed by landfill gas loading. A very interesting observation with implications for methane mitigation was that landfill loading and subsequent gas production was the primary factor governing the performance of passively aerated biofilters. Microbial methane oxidation was limited by outflowing biogas as it prevented diffusion of atmospheric oxygen into the biofilter.

A number of full-scale biobased research projects are underway in USA, Germany, Denmark, Australia and Canada. In Germany, the MiMethox (Microbial Methane Oxidation in landfill covers) developed a biocover system to reduce the methane emitted from landfills generating low-quality biogas. In Canada and Australia, biofilter test cells of different layering and materials have been constructed on landfills to evaluate the methane abatement under Nordic and arid climatic conditions, respectively. In the US, research towards applying biotarps, instead of daily topical applications of soil and wood chips, is underway for methane mitigation. The increasing use of gas collection systems bodes well for biofilters, their small footprint and high removal capacity. The IPCC 2007 assessment report lists biocovers and biofilters as key mitigation technologies that are projected to be commercialised before 2030.

2.3 Denitrification

The interaction between methane and nitrogen has been identified as one of the major gaps in carbon–nitrogen cycle interactions (Gärdenäs et al. 2011; Stein et al. 2012). Methanotrophs and autotrophic nitrifiers share many similarities. Methane oxidisers

and ammonium oxidisers are proposed to have a common evolutionary history as the enzyme systems are similar and the bacteria occupy similar ecological niches (Holmes et al. 1999; Stein et al. 2012). Genes that encode for pMMO or ammonia monooxygenase share high sequence similarities and, despite their different physiological roles, appear to be evolutionarily related enzymes (Holmes et al. 1999).

Methanotrophs can directly or indirectly participate in denitrification, especially in wastewater treatment systems. Modern wastewater treatment systems frequently supplement with costly external carbon sources, such as methanol, to achieve more stringent N discharge limits (Strong et al. 2011). Using methane as a low-cost carbon source to facilitate denitrification would be highly beneficial (Modin et al. 2007). Incorporating methane into the denitrification process was suggested by various researchers in the 1970s (Harremoes and Henze Christensen 1971; Davies 1973; Mason 1977), and four decades later, there have been striking discoveries and substantial progress regarding this coupled process. Methane-dependent denitrification can be divided into two categories according to oxygen availability: aerobic methane oxidation coupled to denitrification (AME-D) or anaerobic methane oxidation coupled to denitrification (ANME-D) (Modin et al. 2007). In spite of the functional differences between the microorganisms responsible for these two processes, the inherent mechanism is dependent on both microbes.

As alternatives are investigated to enable cheaper wastewater denitrification, there has been a recent increase in research published regarding aerobic methane oxidation coupled to denitrification (Zhu et al. 2011; Long et al. 2013; Sun et al. 2013; Liu et al. 2014). It simultaneously ameliorates two environmental issues: methane emissions and soluble nitrogen content in wastewaters. Methane-dependent denitrification appears to be an economical and environment-friendly technology to enable denitrification of nitrogen-contaminated wastewaters (including landfill leachate) by mixed microbial cultures using a cheap, sustainable carbon source (Long et al. 2013; Sun et al. 2013).

2.3.1 Aerobic Methane Oxidation Coupled to Denitrification

As early as the 1970s, it was hypothesised that the responsible agent in the mixed methanotrophic culture was a denitrifying methanol-consuming bacteria that used a methanotroph by-product to perform the initial reduction of nitrate to nitrite. Since then, AME-D has become an attractive focus for both atmospheric methane mitigation and nitrogen removal in wastewater treatment. Although the detailed process mechanisms remain unclear, two main pathways have been proposed. The first mechanism is direct nitrate/nitrite reduction by aerobic methanotrophic bacteria. Although no aerobic methanotroph has demonstrated ability of complete denitrification (i.e. releasing N_2 as the terminal product), partial denitrification is possible. Certain aerobic methanotrophs can produce substantial amounts of nitrous oxide when exposed to high nitrite concentrations (Nyerges et al. 2010), and some of these methanotrophs contain functional denitrification genes (Stein and Klotz 2011). Very recently, *Methylomonas denitrificans* FJG1 directly reduced nitrate to

nitrous oxide (incomplete denitrification) under hypoxic conditions with nitrate as the electron acceptor and methane as the electron donor (Kits et al. 2015). In natural habitats such as lake sediments, incomplete denitrification can be performed by the cooperation of different types of aerobic methanotrophs with one or two denitrifying genes. Incomplete denitrification in the sediment of Lake Dagow (Brandenburg Germany) was initially catalysed by *Methylobacter tundripaludum* (*narG* and *nirS* genes) and completed by *Methylomonas methanica* or *Methylomicrobium alcaliphilum* (*norB* gene) (Dumont et al. 2013).

The second mechanism is indirect denitrification. Here, methanotrophs release soluble organic metabolites (methanol, formaldehyde, formate, acetate, etc.) that provide an electron donor for denitrifying bacteria (Modin et al. 2007). In wastewater treatment systems, nitrate/nitrite reduction is achieved by a consortium of aerobic methanotrophs and denitrifying bacteria. This syntrophic relationship, where one organism lives off the products of another organism, has been verified. Denitrifiers isolated from a methanotrophic environment exposed to an oxygen gradient were able to use methanol, formaldehyde and formate (i.e. methane oxidation intermediates) to achieve denitrification (Knowles 2005). Additionally, methanol- and acetate-consuming denitrifiers performed the denitrification in earlier research, where denitrification was achieved with methane as the carbon source under microaerophilic conditions (Costa et al. 2000).

2.3.2 Anaerobic Methane Oxidation Coupled to Denitrification

Nitrite-dependent anaerobic methane oxidation (n-damo) is a recently discovered process that couples anaerobic methane oxidation to nitrite reduction (Raghoebarsing et al. 2006).The novel mechanism for methane-dependent denitrification uses an intra-aerobic denitrification pathway and was performed by a new species with the proposed name: *Methylomirabilis oxyfera* (Ettwig et al. 2010). Even though it exists in a strictly anoxic environment, *M. oxyfera* encodes, transcribes and expresses all genes involved in aerobic methane oxidation. It was hypothesised to produce oxygen required in methane oxidation via dismutation of nitric oxide to dinitrogen gas and oxygen (Ettwig et al. 2010). It may also be a novel pathway to achieve complete denitrification from nitrite, instead of traditional process that requires nitrous oxide reductase. Since its discovery, the ecology of *M. oxyfera* and n-damo process has been intensely studied. The bacterium is widely distributed in sediments (Deutzmann and Schink 2011; Kojima et al. 2012), wetlands (Hu et al. 2014) and wastewater sludge (Luesken et al. 2011). More recently, the n-damo process was coupled with anaerobic ammonium oxidation to remove nitrogen (ammonium and nitrate) with high removal rates (Zhu et al. 2011; Hu et al. 2012; Shi et al. 2013), which has strong potential as a future wastewater nitrogen removal technology.

2.4 Bioremediation of Organic Contaminants

Methanotrophs are useful bioremediation agents because of the broad substrate range of their MMO enzymes, which allows their use in heavy metal removal (Al Hasin et al. 2010) and transformation of organic pollutants (Pandey et al. 2014). The sMMO and pMMO enzymes can transform a variety of hydrocarbons (summarised in Table 2.2), including alkanes, alkenes, alicyclic hydrocarbons,

Table 2.2 Various hydrocarbons that can be oxidised by sMMO and pMMO enzymes and can transform a variety of hydrocarbons

Substrate	sMMO: major reaction products (relative molar proportions)	pMMO: major reaction products
Alkanes		
Methane	Methanol	Methanol
Ethane	Ethanol	Ethanol; Ethanal
Propane	Propan-1-ol (39); propan-2-ol (61)	Propan-1-ol; Propan-2-ol
Butane	Butan-1-ol (54); butan-2-ol (46)	Butan-2-ol
Pentane		Pentan-2-ol
Hexane	Hexan-1-ol (63); hexan-2-ol (37)	
Octane	Octan-1-ol (9); octan-2-ol (91).	
2-Methylpropane	2-Methylpropan-2-ol (70); 2-methylpropan-1-ol (30)	
Alkenes		
Ethene	Epoxyethane	
Propene	Epoxypropane/Propene oxide	Epoxypropane/Propene oxide
But-1-ene	1,2-Epoxybutane	1,2-Epoxybutane; 3-Buten-2-ol
cis-But-2-ene	*cis*-2,3-Epoxybutane (47); *cis-2*- buten-1-ol (53)	*cis*-2,3-Epoxybutane; Crotonaldehyde
trans-But-2-ene	*trans*-2,3-Epoxybutane (27); *trans-2*-buten-1-ol (73)	
1,3-Butadiene		1,2-Epoxybut-3-ene
cis-But-2-ene		*cis*-2,3-Epoxybutane; Crotonaldehyde
trans-But-2-ene		*trans*-2,3-Epoxybutane; Crotyl alcohol; Crotonaldehyde
Alicyclic hydrocarbons		
Cyclohexane	Cyclohexanol	
Methylene cyclohexane	1-Cyclohexane-1-methanol (13.7); methylene cyclohexane oxide (75.8); 4-hydroxymethylene cyclohexane (10.5)	
β-Pinene	6,6-Dimethylbicyclo[3.1.1] hept-2-ene-2-methanol (72.3); β-pinene oxide (27.7)	
Adamantane	1-Adamantol (50); 2-adamantol (50)	

(continued)

Table 2.2 (continued)

Substrate	sMMO: major reaction products (relative molar proportions)	pMMO: major reaction products
Halogenated aliphatics		
Trichloroethene	Formate (35); CO (53); glyoxylate (5); dichloroacetate (5); chloral (6)	
l,l-Dichloroethene	Glycolate (80); dichloroacetaldehyde (3)	
Chlorotrifluoroethylene	Oxalate	
Tribromoethylene	Formate (80); bromal (5)	
Mono-aromatics		
Benzene	Phenol	
Toluene	Benzyl alcohol (60); cresol (40)	
Ethylbenzene	1-Phenylethanol (30); 4-hydroxyethylbenzene (70)	
Styrene	Styrene oxide	
Pyridine	Pyridine *N*-oxide	
Di-aromatics		
Naphthalene	1-Naphthol, 2-naphthol	
Biphenyl	2-Hydroxybiphenyl (9); 3-hydroxybiphenyl (1); 4-hydroxybiphenyl (90)	
2-Hydroxybiphenyl	Dihydroxybiphenyls	
2-Methylbiphenyl	Ring (56) and side chain (44) hydroxylated products	
2-Chlorobiphenyl	Hydroxychlorobiphenyls	
Other compounds		
Diethyl ether	Ethanol (47); ethanal (53)	
Carbon monoxide	Carbon dioxide	

Adapted from Jiang et al. (2010)

aromatic compounds and halogenated aliphatics (Colby et al. 1977; Schuetz et al. 2003; Smith and Dalton 2004). The enzymes can transform C_1-C_8 n-alkanes into 1- and 2-alcohols, terminal alkenes into 1,2-epoxides and diethyl ether into ethanol/ethanal (Colby et al. 1977). Alkanes are hydroxylated mostly at the terminal and sub-terminal positions, while ring hydroxylation of aromatics occurs primarily at the *meta* position. The sMMO oxygenates alkenes to epoxides with retention of stereochemistry around the C=C double bond (Smith and Murrell 2009). Chlorinated compounds that are degradable by MMOs include chloroform (Alvarez-Cohen and McCarty 1991a), trichloroethylene (Alvarez-Cohen and McCarty 1991a, b; Henry and Grbic-Galic 1990, 1991; Koh et al. 1993; Smith et al. 1997), tetrachloro-ethene (Gerritse et al. 1995), hydrochlorofluorocarbons (Chang and Criddle 1995; DeFlaun et al. 1992), dichloroethene (Janssen et al. 1988) and even vinyl chloride (Nelson and Jewell 1993).

The oxidation of these substrates is termed co-metabolism. The broad range of the MMO enzymes allows for the catalysis, but unlike methanol, the oxidised products are essentially of no use to the cells energetically, as these compounds do not regenerate reducing equivalents that the MMO requires to remain functional for methane catalysis. High concentrations of co-substrates can starve the methanotrophs of energy needed to survive. Methane, methanol, formate or nutrients may be added to stimulate the methanotrophs and enhance biodegradation and biotransformation of contaminants. Biostimulation of methanotrophs according to the site-specific needs has even been demonstrated at a field scale *in situ* within contaminated aquifers and soils, and ex situ in bioreactors (McCarty and Semprini 1994; Semprini et al. 1994; Brigmon 2001; Jiang et al. 2010).

A variety of microbes have been genetically engineered to improve their remediative capacities (Morrissey et al. 2002; Liu et al. 2011; Villacieros et al. 2005; Azad et al. 2014). Genetic engineering may further enhance methanotrophs' tolerance to pollutants and degradation potential, the safety and the risk of genetic transfer, but will require close monitoring if applied in the natural environment (Morrissey et al. 2002; Singh 2011; Pandey et al. 2014). Alternatively, methanotroph–plant associations may be worth pursuing to create a stable methanotroph population in a soil environment—in a symbiotic relationship with plant roots. Even if the methanotrophs do not benefit the host greatly (as is normally the case with endophytes providing nutrients or secreting plant growth-promoting factors), as long as they are actively present in the environment it could be considered beneficial (Azad et al. 2014).

Although methanotrophs are capable of environmental detoxification, providing conditions to maintain an introduced methanotrophic culture, or enriching for methanotrophs may be difficult to implement and justify economically over large areas or dilute pollutant concentrations. Environmental remediation seldom has a commercial value other than avoiding enforced penalties, and the methanotrophs have too many specialised requirements to consider the catalytic whole-cell transformation as a useful tool for bioremediation. However, one avenue that could yield positive results without requiring intensive operational monitoring is using the plant–methanotroph symbiont relationship to enhance phytoremediation and bioremediation.

2.5 Conclusion

Methanotrophs are a diverse group of bacteria that are capable of mitigating anthropogenic methane emissions, removing N from environmental and wastewater treatment systems and can even transform organic pollutants in soils. Methanotrophic methane mitigation technologies have been demonstrated beyond the laboratories as adaptable field-scale systems that may be engineered to meet site-specific climatic variations and ensure minimal atmospheric methane emission. However, they are not without their limitations as methane is required to maintain cell activity and large volumes of gas with low methane content can be difficult to treat effectively

and cost efficiently. In agricultural sediments and soils, methanotrophs sequester methane, but are affected by fertiliser applications, while in wastewater treatment systems they can lower the costs associated providing an external carbon source to remove N. Methanotrophs are appealing as bioremediation agents in methane-containing environments, as their primary enzymes have a broad substrate range that can transform various hydrocarbons, including aromatic compounds and halogenated aliphatics. These bacteria are an important global methane sink and their importance will increase as anthropogenic emissions and environmental standards increase over the coming decades.

References

Al Hasin A, Gurman SJ, Murphy LM, Perry A, Smith TJ, Gardiner PHE (2010) Remediation of chromium(VI) by a methane-oxidizing bacterium. Environ Sci Technol 44(1):400–405

Alvarez-Cohen L, McCarty PL (1991a) Product toxicity and cometabolic competitive-inhibition modeling of chloroform and trichloroethylene transformation by methanotrophic resting cells. Appl Environ Microbiol 57:1031–1037

Alvarez-Cohen L, McCarty PL (1991b) Effects of toxicity, aeration, and reductant supply on trichloroethylene transformation by a mixed methanotrophic culture. Appl Environ Microbiol 57(1):228–235

Andreasen RR, Poulsen TG, Iversen N, Roslev P (2013) Stabilization and stimulation of atmospheric methane oxidation in soil and soil biofilters by Al_2O_3 amendment. Soil Biol Biochem 64:127–135

Antony CP, Doronina NV, Boden R, Trotsenko YA, Shouche YS, Murrell JC (2012) Methylophaga lonarensis sp. nov., a moderately haloalkaliphilic methylotroph isolated from the soda lake sediments of a meteorite impact crater. Int J Syst Evol Microbiol 62(7):1613–1618

Azad MA, Amin L, Sidik NM (2014) Genetically engineered organisms for bioremediation of pollutants in contaminated sites. Chin Sci Bull 59(8):703–714

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 13:66

Bodelier PLE, Frenzel P (1999) Contribution of methanotrophic and nitrifying bacteria to CH_4 and NH_4^+ oxidation in the rhizosphere of rice plants as determined by new methods of discrimination. Appl Environ Microbiol 65(5):1826–1833

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiol Ecol 47(3):265–277

Bodelier PL, Roslev P, Henckel T, Frenzel P (2000) Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. Nature 403(6768):421–424

Börjesson G, Sundh I, Svensson B (2004) Microbial oxidation of CH_4 at different temperatures in landfill cover soils. FEMS Microbiol Ecol 48(3):305–312

Brigmon RL (2001) Methanotrophic bacteria: use in bioremediation. U.S. Report No. WSRC-MS-2001-00058 37831–0062. Department of Energy, Oak Ridge

Cabral A, Arteaga K, Rannaud D, Aït-Benichou S, Pouët M, Allaire S, Jugnia L, Greer C (2007) Analysis of methane oxidation and dynamics of methanotrophs within a passive methane oxidation barrier. In: 11th international waste management and landfill symposium, Sta. M. di Pula, Italy

Chang WK, Criddle C (1995) Biotransformation of HCFC-22, HCFC-142b, HCFC-123, and HFC-134a by methanotrophic mixed culture MM1. Biodegradation 6(1):1–9

Chanton JP (2005) The effect of gas transport on the isotope signature of methane in wetlands. Org Geochem 36(5):753–768

Chistoserdova L, Vorholt JA, Lidstrom ME (2005) A genomic view of methane oxidation by aerobic bacteria and anaerobic archaea. Genome Biol 6(2):208

Colby J, Stirling D, Dalton H (1977) The soluble methane monooxygenase of *Methylococcus capsulatus* (Bath): its ability to oxygenate n-alkanes, n-alkenes, ethers, and acyclic, aromatic and heterocyclic compounds. Biochem J 165:395–402

Costa C, Dijkema C, Friedrich M, Garcia-Encina P, Fernandez-Polanco F, Stams AJM (2000) Denitrification with methane as electron donor in oxygen-limited bioreactors. Appl Microbiol Biotechnol 53(6):754–762

Culpepper MA, Rosenzweig AC (2012) Architecture and active site of particulate methane monooxygenase. Crit Rev Biochem Mol Biol 47(6):483–492

Davies TR (1973) Isolation of bacteria capable of utilizing methane as a hydrogen donor in the process of denitrification. Water Res 7(4):575–579

DeFlaun MF, Ensley BD, Steffan RJ (1992) Biological oxidation of hydrochlorofluorocarbons (HCFCs) by a methanotrophic bacterium. Nat Biotechnol 10(12):1576–1578

Delgado JA, Mosier AR (1996) Mitigation alternatives to decrease nitrous oxides emissions and urea-nitrogen loss and their effect on methane flux. J Environ Qual 25(5):1105–1111

Deutzmann JS, Schink B (2011) Anaerobic oxidation of methane in sediments of Lake Constance, an oligotrophic freshwater lake. Appl Environ Microbiol 77(13):4429–4436

Dever SA, Swarbrick GE, Stuetz RM (2007) Passive drainage and biofiltration of landfill gas: Australian field trial. Waste Manag 27(2):277–286

Dever SA, Swarbrick GE, Stuetz RM (2011) Passive drainage and biofiltration of landfill gas: results of Australian field trial. Waste Manag 31(5):1029–1048

Dijk J, Huizing HJ, de Vries C, Bone LJ (2012) An investigation into novel concepts to remove GHG methane from dairy stable ventilation air at ultra-low concentrations by the use of methanotrophic bacteria. Innovation Network Reinvent Agribusiness Rural Areas, Netherlands 29

Dumont MG, Pommerenke B, Casper P (2013) Using stable isotope probing to obtain a targeted metatranscriptome of aerobic methanotrophs in lake sediment. Environ Microbiol Rep 5(5):757–764

Dunfield PF, Khmelenina VN, Suzina NE, Trotsenko YA, Dedysh SN (2003) Methylocella silvestris sp. nov., a novel methanotroph isolated from an acidic forest cambisol. Int J Syst Evol Microbiol 53(5):1231–1239

Einola JKM, Kettunen RH, Rintala JA (2007) Responses of methane oxidation to temperature and water content in cover soil of a boreal landfill. Soil Biol Biochem 39(5):1156–1164

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MM, Schreiber F, Dutilh BE, Zedelius J, de Beer D, Gloerich J, Wessels HJ, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HJ, Janssen-Megens EM, Francoijs KJ, Stunnenberg H, Weissenbach J, Jetten MS, Strous M (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464(7288):543–548

Feig AL, Lippard SJ (1994) Reactions of non-heme iron(II) centers with dioxygen in biology and chemistry. Chem Rev 94(3):759–805

Gardenas AI, Agren GI, Bird JA, Clarholm M, Hallin S, Ineson P, Katterer T, Knicker H, Nilsson SI, Nasholm T, Ogle S, Paustian K, Persson T, Stendahl J (2011) Knowledge gaps in soil carbon and nitrogen interactions—from molecular to global scale. Soil Biol Biochem 43(4):702–717

Gebert J, Groengroeft A, Miehlich G (2003) Kinetics of microbial landfill methane oxidation in biofilters. Waste Manag 23(7):609–619

Gebert J, Singh BK, Pan Y, Bodrossy L (2009) Activity and structure of methanotrophic communities in landfill cover soils. Environ Microbiol Rep 1(5):414–423

Gerritse J, Renard V, Visser J, Gottschal JC (1995) Complete degradation of tetrachloroethene by combining anaerobic dechlorinating and aerobic methanotrophic enrichment cultures. Appl Microbiol Biotechnol 43(5):920–928

Hackl E, Zechmeister-Boltenstern S, Bodrossy L, Sessitsch A (2004) Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Appl Environ Microbiol 70(9):5057–5065

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60(2):439–471

Harremoes P, Henze Christensen M (1971) Denitrification with methane (Denitrifikation med methan). Vand 1:7–11

Haubrichs R, Widmann R (2006) Evaluation of aerated biofilter systems for microbial methane oxidation of poor landfill gas. Waste Manag 26(4):408–416

He R, Ruan A, Jiang C, Shen DS (2008) Responses of oxidation rate and microbial communities to methane in simulated landfill cover soil microcosms. Bioresour Technol 99(15):7192–7199

Heimann K, Karthikayan OP, Cires S (2013) A biotechnological approach to mitigate green house gas emissions from coal mine ventilation air in Australia. In: 1st international conference on technologies for sustainable waste management in developing countries, 23–24 August 2013, Guntur, India

Henry SM, Grbic-Galic D (1990) Effect of mineral media on trichloroethylene oxidation by aquifer methanotrophs. Microb Ecol 20(1):151–169

Henry SM, Grbic-Galic D (1991) Influence of endogenous and exogenous electron donors and trichloroethylene oxidation toxicity on trichloroethylene oxidation by methanotrophic cultures from a groundwater aquifer. Appl Environ Microbiol 57(1):236–244

Hirayama H, Suzuki Y, Abe M, Miyazaki M, Makita H, Inagaki F, Uematsu K, Takai K (2011) Methylothermus subterraneus sp. nov., a moderately thermophilic methanotroph isolated from a terrestrial subsurface hot aquifer. Int J Syst Evol Microbiol 61(11):2646–2653

Ho A, de Roy K, Thas O, De Neve J, Hoefman S, Vandamme P, Heylen K, Boon N (2014) The more, the merrier: heterotroph richness stimulates methanotrophic activity. ISME J 8(9): 1945–1948

Holmes AJ, Roslev P, McDonald IR, Iversen N, Henriksen K, Murrell JC (1999) Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. Appl Environ Microbiol 65(8):3312–3318

Hu ZY, Speth DR, Francoijs KJ, Quan ZX, Jetten MSM (2012) Metagenome analysis of a complex community reveals the metabolic blueprint of anannmox bacterium "Candidatus Jettenia asiatica". Front Microbiol 3:9

Hu BL, Shen LD, Lian X, Zhu Q, Liu S, Huang Q, He ZF, Geng S, Cheng DQ, Lou LP, Xu XY, Zheng P, He YF (2014) Evidence for nitrite-dependent anaerobic methane oxidation as a previously overlooked microbial methane sink in wetlands. Proc Natl Acad Sci U S A 111(12):4495–4500

Huber-Humer M, Gebert J, Hilger H (2008) Biotic systems to mitigate landfill methane emissions. Waste Manag Res 26(1):33–46

Humer M, Lechner P (1999) Alternative approach to the elimination of greenhouse gases from old landfills. Waste Manag Res 17(6):443–452

Hütsch BW, Webster CP, Powlson DS (1994) Methane oxidation in soil as affected by land use, soil pH and N fertilization. Soil Biol Biochem 26(12):1613–1622

IPCC (2001) The scientific basis. In: Houghton JT et al (eds) Contribution of working group I to the third assessment report of the intergovernmental panel on climate change. Cambridge University Press, New York

IPCC (2013) IPCC Fifth Assessment Report (AR4). Climate change 2013: the physical science basis. In: Stocker TF, Qin D (eds) Working group I contribution to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, pp 93–129

IRRI (2015) Greenhouse gas mitigation in irrigated rice systems in Asia. http://climatechange.irri.org/projects/mitigation-projects/mirsa

Janssen D, Grobben G, Hoekstra R, Oldenhuis R, Witholt B (1988) Degradation of trans-1,2-dichloroethene by mixed and pure cultures of methanotrophic bacteria. Appl Microbiol Biotechnol 29(4):392–399

Jiang H, Chen Y, Jiang P, Zhang C, Smith TJ, Murrell JC, Xing XH (2010) Methanotrophs: multifunctional bacteria with promising applications in environmental bioengineering. Biochem Eng J 49(3):277–288

Kalyuzhnaya MG, Stolyar SM, Auman AJ, Lara JC, Lidstrom ME, Chistoserdova L (2005) Methylosarcina lacus sp. nov., a methanotroph from Lake Washington, Seattle, USA, and emended description of the genus Methylosarcina. Int J Syst Evol Microbiol 55(6): 2345–2350

Kalyuzhnaya MG, Puri AW, Lidstrom ME (2015) Metabolic engineering in methanotrophic bacteria. Metab Eng 29:142–152

Karthikeyan O, Karthigeyan C, Cires S, Heimann K (2015) Review of sustainable methane mitigation and bio-polymer production. Crit Rev Environ Sci Technol 45(15):1579–1610

Kemnitz D, Kolb S, Conrad R (2005) Phenotypic characterization of Rice Cluster III archaea without prior isolation by applying quantitative polymerase chain reaction to an enrichment culture. Environ Microbiol 7(4):553–565

Kim HG, Han GH, Eom CY, Kim SW (2008) Isolation and taxonomic characterization of a novel type I methanotrophic bacterium. J Microbiol 46(1):45–50

Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, Bergamaschi P, Bergmann D, Blake DR, Bruhwiler L (2013) Three decades of global methane sources and sinks. Nat Geosci 6(10):813–823

Kits KD, Klotz MG, Stein LY (2015) Methane oxidation coupled to nitrate reduction under hypoxia by the Gammaproteobacterium Methylomonas denitrificans, sp. nov. type strain FJG1. Environ Microbiol 17(9):3219–3232

Knief C, Lipski A, Dunfield PF (2003) Diversity and activity of methanotrophic bacteria in different upland soils. Appl Environ Microbiol 69(11):6703–6714

Knittel K, Boetius A (2009) Anaerobic oxidation of methane: progress with an unknown process. Annu Rev Microbiol 63:311–334

Knowles R (2005) Denitrifiers associated with methanotrophs and their potential impact on the nitrogen cycle. Ecol Eng 24(5):441–446

Koh SC, Bowman JP, Sayler GS (1993) Soluble methane monooxygenase production and trichloroethylene degradation by a Type I methanotroph, Methylomonas methanica 68-1. Appl Environ Microbiol 59(4):960–967

Kojima H, Tsutsumi M, Ishikawa K, Iwata T, Mussmann M, Fukui M (2012) Distribution of putative denitrifying methane oxidizing bacteria in sediment of a freshwater lake, Lake Biwa. Syst Appl Microbiol 35(4):233–238

Kravchenko I, Boeckx P, Galchenko V, Van Cleemput O (2002) Short- and medium-term effects of NH4+ on CH_4 and N_2O fluxes in arable soils with a different texture. Soil Biol Biochem 34(5):669–678

Kubo M, Purevdorj M (2004) The future of rice production and consumption. J Food Distrbut Res 35((1)):128–142

Lakshmanan A, Raj SA, Kareem AA (1994) Biofertilizers enhance dissolved oxygen content in water. Crop Res 82(2):283–286

Lakshmi VG, Lakshmanan A, Sankar A, Latha P, Udaya SN (2012) Biofertilizers in minimizing climate change impacts in rice farming. Climarice 9:1–7

Lee EH, Moon KE, Ryu HW (2011) Characterization of methane oxidation by a methanotroph isolated from a landfill cover soil, South Korea. J Microbiol Biotechnol 21(7):753–756

Limbri H, Gunawan C, Rosche B, Scott J (2013) Challenges to developing methane biofiltration for coal mine ventilation air: a review. Water Air Soil Pollut 224(6):1–15

Limbri H, Gunawan C, Thomas T, Smith A, Scott J, Rosche B (2014) Coal-packed methane biofilter for mitigation of green house gas emissions from coal mine ventilation air. PLoS One 9(4):e94641

Liu S, Zhang F, Chen J, Sun GX (2011) Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions. J Environ Sci 23(9):1544–1550

Liu JJ, Sun FQ, Wang L, Ju X, Wu WX, Chen YX (2014) Molecular characterization of a microbial consortium involved in methane oxidation coupled to denitrification under micro-aerobic conditions. J Microbial Biotechnol 7(1):64–76

Long Y, Zhong Z, Yin H, Lin Z, Ye J, He B (2013) Characteristic of methane oxidation coupled to denitrification in cover soils of landfill. Trans Chin Soc Agric Eng 29(15):207–214

Luesken FA, van Alen TA, van der Biezen E, Frijters C, Toonen G, Kampman C, Hendrickx TL, Zeeman G, Temmink H, Strous M, Op den Camp HJ, Jetten MS (2011) Diversity and enrichment of nitrite-dependent anaerobic methane oxidizing bacteria from wastewater sludge. Appl Microbiol Biotechnol 92(4):845–854

Mandal LN, Mitra RR (1982) Transformation of iron and manganese in rice soils under different moisture regimes and organic matter applications. Plant and Soil 69(1):45–56

Mason I (1977) Methane as a carbon source in biological denitrification. J Water Pollut Control Fed 49(5):855–857

McCarty PL, Semprini L (1994) Ground-water treatment for chlorinated solvents. In: Matthews JE (ed) Handbook of bioremediation. Lewis Publishers, Boca Raton, FL, pp 87–116

McDonald IR, Bodrossy L, Chen Y, Murrell JC (2008) Molecular ecology techniques for the study of aerobic methanotrophs. Appl Environ Microbiol 74(5):1305–1315

Modin O, Fukushi K, Yamamoto K (2007) Denitrification with methane as external carbon source. Water Res 41(12):2726–2738

Mohanty SR, Bodelier PLE, Floris V, Conrad R (2006) Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. Appl Environ Microbiol 72(2):1346–1354

Morrissey JP, Walsh UF, O'Donnell A, Moenne-Loccoz Y, O'Gara F (2002) Exploitation of genetically modified inoculants for industrial ecology applications. Antonie Van Leeuwenhoek 81(1–4):599–606

Nelson Y, Jewell W (1993) Vinyl chloride biodegradation with methanotrophic attached films. J Environ Eng 119(5):890–907

Nikiema J, Brzezinski R, Heitz M (2007) Elimination of methane generated from landfills by biofiltration: a review. Rev Environ Sci Biotehnol 6(4):261–284

Nyerges G, Han SK, Stein LY (2010) Effects of ammonium and nitrite on growth and competitive fitness of cultivated methanotrophic bacteria. Appl Environ Microbiol 76(16):5648–5651

Pandey VC, Singh JS, Singh DP, Singh RP (2014) Methanotrophs: promising bacteria for environmental remediation. Int J Environ Sci Technol 11(1):241–250

Park S, Brown KW, Thomas JC (2002) The effect of various environmental and design parameters on methane oxidation in a model biofilter. Waste Manag Res 20(5):434–444

Park S, Lee CH, Ryu CR, Sung K (2009) Biofiltration for reducing methane emissions from modern sanitary landfills at the low methane generation stage. Water Air Soil Pollut 196(1–4):19–27

Pfluger AR, Wu WM, Pieja AJ, Wan J, Rostkowski KH, Criddle CS (2011) Selection of Type I and Type II methanotrophic proteobacteria in a fluidized bed reactor under non-sterile conditions. Bioresour Technol 102(21):9919–9926

Pingak GMF, Sutanto H, Akhdiya A, Rusmana I (2014) Effectivity of methanotrophic bacteria and *Ochrobactrum anthropi* as biofertilizer and emission reducer of CH_4 and N_2O in inorganic paddy fields. J Med Bioeng 3(3):217–221

Prasanna R, Kumar V, Kumar S, Yadav A, Tripathi U, Kumar Singh A, Jain MC, Gupta P, Singh PK, Sethunathan N (2002) Methane production in rice soil is inhibited by cyanobacteria. Microbiol Res 157(1):1–6

Rachor I, Gebert J, Groengroeft A, Pfeiffer EM (2011) Assessment of the methane oxidation capacity of compacted soils intended for use as landfill cover materials. Waste Manag 31(5):833–842

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJ, Ettwig KF, Rijpstra WI, Schouten S, Damste JS, Op den Camp HJ, Jetten MS, Strous M (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440(7086):918–921

Raja N (2013) Biopesticides and bio-fertilizers: eco-friendly sources for sustainable agriculture. Biofert Biopest 4:1–2

Reeburgh WS (2003) Global methane biogeochemistry. In: Ralph FK, Heinrich DH, Karl K (eds) Treatise on geochemistry. Elsevier, 89, p 65

Reeburgh WS, Whalen SC, Alperin MJ (1993) The role of methylotrophy in the global methane budget. In: Murrell JC, Kelly DP (eds) Microbial growth on C1 compounds. Intercept Limited, Andover, pp 1–14

Rösch C, Mergel A, Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68(8):3818–3829

Sadasivam BY, Reddy KR (2015) Adsorption and transport of methane in landfill cover soil amended with waste-wood biochars. J Environ Manage 158:11–23

Sahoo RK, Ansari MW, Pradhan M, Dangar TK, Mohanty S, Tuteja N (2014) Phenotypic and molecular characterization of native *Azospirillum* strains from rice fields to improve crop productivity. Protoplasma 251(4):943–953

Saidi-Mehrabad A, He Z, Tamas I, Sharp CE, Brady AL, Rochman FF, Bodrossy L, Abell GC, Penner T, Dong X (2013) Methanotrophic bacteria in oilsands tailings ponds of northern Alberta. ISME J 7(5):908–921

Schimel J (2000) Global change: rice, microbes and methane. Nature 403(6768):375–377

Schuetz C, Bogner J, Chanton J, Blake D, Morcet M, Kjeldsen P (2003) Comparative oxidation and net emissions of methane and selected non-methane organic compounds in landfill cover soils. Environ Sci Technol 37(22):5150–5158

Seghers D, Top EM, Reheul D, Bulcke R, Boeckx P, Verstraete W, Siciliano SD (2003) Long-term effects of mineral versus organic fertilizers on activity and structure of the methanotrophic community in agricultural soils. Environ Microbiol 5(10):867–877

Semprini L, Hopkins GD, Grbic-Galic D, McCarthy PL, Roberts PV (1994) A laboratory and field evaluation of enhanced in situ bioremediation of trichloroethylene, cis- and trans-dichloroethylene, and vinyl chloride by methanotrophic bacteria. In: Flathman PE, Jerger DE, Exner JG (eds) Bioremediation: field experience. CRC Press, Boca Raton, FL, pp 383–412

Semrau JD, DiSpirito AA, Yoon S (2010) Methanotrophs and copper. FEMS Microbiol Rev 34(4):496–531

Sharp CE, Smirnova AV, Graham JM, Stott MB, Khadka R, Moore TR, Grasby SE, Strack M, Dunfield PF (2014) Distribution and diversity of *Verrucomicrobia* methanotrophs in geothermal and acidic environments. Environ Microbiol 16(6):1867–1878

Shi Y, Hu SH, Lou JQ, Lu PL, Keller J, Yuan ZG (2013) Nitrogen removal from wastewater by coupling anammox and methane-dependent denitrification in a membrane biofilm reactor. Environ Sci Technol 47(20):11577–11583

Shiemke AK, Cook SA, Miley T, Singleton P (1995) Detergent solubilization of membrane-bound methane monooxygenase requires plastoquinol analogs as electron donors. Arch Biochem Biophys 321(2):421–428

Singh JS (2011) Methanotrophs: the potential biological sink to mitigate the global methane load. Curr Sci 100(1):29–30

Singh JS, Pandey VC (2013) Fly ash application in nutrient poor agriculture soils: impact on methanotrophs population dynamics and paddy yields. Ecotoxicol Environ Saf 89:43–51

Singh JS, Strong PJ (2015) Biologically derived fertilizer: a multifaceted bio-tool in methane mitigation. Ecotoxicol Environ Saf 124:267–276

Singh JS, Pandey VC, Singh DP, Singh RP (2010) Influence of pyrite and farmyard manure on population dynamics of soil methanotroph and rice yield in saline rain-fed paddy field. Agric Ecosyst Environ 139(1–2):74–79

Sly LI, Bryant LJ, Cox JM, Anderson JM (1993) Development of a biofilter for the removal of methane from coal mine ventilation atmospheres. Appl Microbiol Biotechnol 39(3):400–404

Smith TJ, Dalton H (2004) Biocatalysis by methane monooxygenase and its implications for the petroleum industry. In: Vazquez-Duhalt R, Quintero-Ramirez R (eds) Petroleum biotechnology—developments and perspectives, vol 151. Elsevier Science, Amsterdam, pp 177–192

Smith TJ, Murrell JC (2009) Methanotrophs: biotechnological potential and emerging applications. In: Flickinger M (ed) Encyclopedia of industrial biotechnology. Willey, New York

Smith TJ, Murrell JC (2010) Methanotrophs. In: Flickenger MC (ed) Encyclopedia of industrial biotechnology. Wiley, Hoboken, NJ, pp 1–13

Smith KS, Costello AM, Lidstrom ME (1997) Methane and trichloroethylene oxidation by an estuarine methanotroph, *Methylobacter* sp. strain BB5.1. Appl Environ Microbiol 63(11): 4617–4620

Stein LY, Klotz MG (2011) Nitrifying and denitrifying pathways of methanotrophic bacteria. Biochem Soc Trans 39:1826–1831

Stein L, Roy R, Dunfield P (2012) Aerobic methanotrophy and nitrification: processes and connections. Encyclopedia of life sciences. Wiley, Chichester

Strong PJ, McDonald B, Gapes DJ (2011) Enhancing denitrification using a carbon supplement generated from the wet oxidation of waste activated sludge. Bioresour Technol 102(9): 5533–5540

Strong P, Xie S, Clarke WP (2015) Methane as a resource: can the methanotrophs add value? Environ Sci Technol 49(7):4001–4018

Su S, Beath A, Guo H, Mallett C (2005) An assessment of mine methane mitigation and utilisation technologies. Prog Energ Combust Sci 31:123–170

Sun FY, Dong WY, Shao MF, Lv XM, Li J, Peng LY, Wang HJ (2013) Aerobic methane oxidation coupled to denitrification in a membrane biofilm reactor: treatment performance and the effect of oxygen ventilation. Bioresour Technol 145:2–9

Villacieros M, Whelan C, Mackova M, Molgaard J, Sanchez-Contreras M, Lloret J, de Carcer DA, Oruezabal RI, Bolanos L, Macek T, Karlson U, Dowling DN, Martin M, Rivilla R (2005) Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 derivatives, using a *Sinorhizobium meliloti* nod system to drive bph gene expression. Appl Environ Microbiol 71(5):2687–2694

Wendlandt KD, Stottmeister U, Helm J, Soltmann B, Jechorek M, Beck M (2010) The potential of methane-oxidizing bacteria for applications in environmental biotechnology. Eng Life Sci 10(2):87–102

Yadav RK, Abraham G, Singh YV, Singh PK (2014) Advancements in the utilization of Azolla-Anabaena system in relation to sustainable agricultural practices. Proc Indian Natl Sci Acad 80(2):301–316

Zheng Y, Liu X, Zhang L, Zhou Z, He J (2010) Do land utilization patterns affect methanotrophic communities in a Chinese upland red soil? J Environ Sci 22(12):1936–1943

Zhu B, Sanchez J, van Alen TA, Sanabria J, Jetten MSM, Ettwig KF, Kartal B (2011) Combined anaerobic ammonium and methane oxidation for nitrogen and methane removal. Biochem Soc Trans 39:1822–1825

Chapter 3
Prospects of Bacterial-Assisted Remediation of Metal-Contaminated Soils

Muhammad Saleem, Hafiz Naeem Asghar, Waqar Ahmad, Muhammad Ahmed Akram, Muhammad Usman Saleem, Muhammad Yahya Khan, Muhammad Naveed, and Zahir Ahmad Zahir

Abstract Industrial revolution resulted in plenty of contaminants in the environment. Several organic and inorganic pollutants have adversely affected soils and water resources, causing serious health issues in humans. Among inorganic contaminants heavy metals are of prime importance as they are nondegradable in the environment. Arsenic, cadmium, chromium, cobalt, copper, lead, mercury, selenium, zinc, and other metals originating from various point and nonpoint sources are contaminating natural resources. Elevated concentrations of poisonous metals are not only disturbing soil health and microbial ecology but also decreasing crop production and global food security. Entry of metal pollutants into the food chain is dangerous for human health. Serious efforts are needed to mitigate rising threats of metal contamination. Physical, chemical, and biological approaches can be used to remediate such type of pollutants. However, bioremediation is considered as a promising technique, being cost effective and environment friendly with minimum adverse effects, esthetic advantages, and long-term applicability. Phytoremediation is a type of bioremediation to remove toxic metals from soil through hyperaccumulation or phytostabilization in plant cells. Generally, higher contents of toxic metals in soil and water result in more uptake by roots and more translocation toward shoots, causing interference in metabolism and reduced growth. Successful phytoremediation is limited to the plant types, tolerance to the high metal concentrations, accumulation rate, growth rate, adaptability, and biomass production. Metal-tolerant bacteria can help plant to tolerate metal stress via different mechanisms involved including

M. Saleem • H.N. Asghar (✉) • W. Ahmad • M.A. Akram • M.U. Saleem
M. Naveed • Z.A. Zahir
Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan
e-mail: naeemasghar@yahoo.com

M.Y. Khan
Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

University of Agriculture, Faisalabad, Sub-Campus Burewala, Vehari, Pakistan

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_3

production of different hormones such as auxins, cytokinin, and gibberellic acid or suppressing stress-induced enzymes such as plant ethylene level. This chapter reviews possible interactions between plant and bacteria to make situations more conducive for remediation of metal-contaminated soil. The chapter also covers different strategies/mechanisms adopted by plants and bacteria to mitigate toxic effect of metals on plant growth in metal-contaminated soils.

Keywords Bioremediation • Hyperaccumulators • Phytoremediation • Soil pollution

3.1 Introduction

Modernization has eased human life at the cost of enormous environmental pollution. Human settlements in urban areas and industrial growth have contributed majorly to the environmental concerns. In developing countries where industrial growth has become a prime focus and agricultural economics has been neglected, rural to urban shift has direct implications on soil, air, and water pollutions. Consequently, human health has to face various challenges due to the release of xenobiotics, pollutants, and heavy metals in the environment through industrial effluents. Many of the industries use heavy metals and their effluents containing significant concentrations of heavy metals are dumped without any treatment. The heavy metals released by the industries are deteriorating our soil and water resources. Entry of heavy metals in the food chain can be drastic as many of these heavy metals are carcinogenic to the human. Keeping in view the release of heavy metals to the environment and their associated threats, strategies to rehabilitate our environment must be devised. Microbial-based bioremediation has been considered as promising, cost effective, and environment friendly technique to decontaminate the heavy metals and other toxic compounds from environment (Singh et al. 2011). To understand the gravity of this problem and to cope with the possible consequences of heavy metal pollution, a comprehensive understanding needs to be developed in masses regarding the mechanisms and roles these heavy metals are playing in deterioration of environment and human health.

3.2 Heavy Metals as Soil Pollutants

Heavy metals are the transitional elements with densities higher than 5 g cm^{-3}. Metals such as Iron (Fe), Zinc (Zn), Lead (Pb), Cadmium (Cd), and Mercury (Hg) are some examples of heavy metals. Heavy metal pollution is a major cause of environmental instability, due to their extensive use, distribution, and toxicity to the human. However, low concentrations of some elements such as Iron (Fe) and Zinc (Zn) are required for the proper functioning of human body (Rouphael et al. 2008).

3.3 Sources of Heavy Metals

Metals in soil can accumulate naturally as well as by anthropogenic activities. Weathering of metal rocks, translocation of main land dust particles, and atmospheric secretions from volcanoes are the natural sources of heavy metal release into the environment. Whereas, anthropogenic sources of metal release into soil, water, and environment include exploitation of minerals through mining, agricultural utilization of sewage sludge as organic matter, increased use of electric appliances, metal consumption in industrial process, burning of metal-supplemented fossil fuel in vehicles, and increased reliance on military training to ensure countries defense. The chief man-made sources of heavy metal contamination to the soil are the application of untreated sludge to agricultural lands and industrialization (Shi et al. 2005).

Although industrial effluents are the main cause of heavy metal pollution, yet the domestic waste water also provide significant contribution in this kind of pollution. Agricultural soils in the close proximity of industries are highly polluted with heavy metal; however, the heavy metal pollution has also been found in the suburban to rural areas, where the injudicious use of pesticides, fertilizers, and irrigation with polluted water have contributed to the accumulation of heavy metals in the soil. Industrial wastes are discharged into the rivers, canal, and other water bodies without any sort of treatment. These metals when taken up by the human can be fatal and in acute case death may occur (Sanayei et al. 2009).

3.4 Heavy Metal Concentration

Rising infestation of heavy metals in the environment has hazardous influences on human health and agriculture. In the industrial cities concentrations of Hg, Cd, Pb, As, and Ni already have crossed the permissible limits in soil. In the various geological regions of world, the concentrations of these metals vary from less than 1 to 100,000 ppm. Whereas, permissible limits in the soil are 4960, 120, 480, 810, 460, and 410 ppm for tin, chromium, lead, manganese, nickel, and copper, respectively (Binggan and Yang 2010). The variability in the heavy metal contamination can be due to different agronomic practices. Increased use of phosphate fertilizers and pesticides may also be one reason of contamination of heavy metals in soil (Tumuklu et al. 2007).

3.5 Toxic Effects of Heavy Metals

Plants uptake ions present in the soil solution and utilize them in their metabolism. Simultaneously, nonspecific absorption of soluble heavy metals also occurs. Most of the heavy metals are nonessential for plants and are compartmentalized in the

plant tissues (Mohammad et al. 2003). Accumulation of heavy metals is prominent in the crops growing near the industrial areas. Heavy metal exposure to plants at lower concentrations for long duration causes functional syndrome in plants and human. However, metal toxicity accompanied by oxidative stress is caused by high concentrations (John et al. 2009). Production of reactive oxygen species such as superoxide (O_2^-), singlet oxygen (O^-), hydrogen peroxide (H_2O_2), and hydroxyl ions (OH^-) due to the oxidative stress generated by the heavy metals causes disintegration of cell membranes, imparts cell functioning, and eventually leads to cell death in plants. Bioaccumulation of heavy metals substitutes different enzymes and metals of prime importance by fostering oxidative stress which causes disruption of different functions. It also affects the plant growth by hindering the photosynthetic activity which causes senescence. Heavy metals are more toxic when they are present in their elemental or chemically combined state. Response of plants to these toxic metals depends on their nature and differs from species to species (Talanova et al. 2000). Metal such as cadmium (Cd) reduces the uptake of essential nutrients, decreases the photosynthetic activity, and slows down the plant growth. Reactive oxygen species are produced due to oxidative stress caused by mercury (Hg^{+2}) that disturbs the mitochondrial activity and lead (Pb) at elevated levels dismantle mineral nutrition inhibiting the enzyme activity, causes water imbalance, and alters membrane permeability. In terms of growth, seedling is more susceptible to heavy metal toxicity as compared to seed germination. Moreover, heavy metal toxicity also disturbs many physiological processes such as photosynthesis, transpiration, and enzymatic activity of plants. Various researchers have investigated the harmful effects of heavy metals; Oancea et al. (2005) concluded retardation in growth of tomato and structural damage due to Cr, Hg, Cd, and Zn toxicity; these metals also effected the physiological and biochemical activities of tested plants. Weiqiang et al. (2005) compared the growth of seedlings and seed germination in heavy metals toxicity and found that seedling growth was more susceptible to heavy metal toxicity as compared to seed germination Tuna et al. (2002) evaluated heavy metal toxicity on germination and pollen tubes in tobacco plant (*Nicotiana tabacum* L.). Outcomes of the experiment showed that with the increasing concentration of heavy metal the pollen length was decreased. Peralta et al. (2004) checked different concentrations of heavy metals including Cd, Cu, Ni on growth of alfalfa (*Medicago sativa*) plants. Various concentrations of metals, viz., 0, 5, 10, 20, and 40 ppm were used. Results showed that Cd strongly affected the germination and growth of seeds at 10 ppm, while Cu and Ni at 20 ppm and higher concentrations. It has also been learnt that seed germination was not affected by Zn. Gopal and Khurana (2011) tested different heavy metals (Pb, Cr, Cu, Ni, and Cd) on plant growth and stress symptoms were visible at 0.25 mM of metal in soil. It was also observed that these heavy metals decreased leaf mass, plant height, growth, affected enzymatic activity, head size of flowers, delayed flowering, and also caused interveinal chlorosis.

3.6 Techniques Used for Remediation of Metal-Contaminated Soils

Different approaches are used to remediate the metal-contaminated soils. The choice of these approaches relies on the contaminant nature, cost of technologies, characteristics of sites, and time.

3.6.1 Physicochemical Techniques

Physicochemical methods used for remediation of heavy metals involved the following.

3.6.1.1 Isolation

In this technique, heavy metals movement is restricted or metals mobility is prevented. For this technique, physical barriers are used to prevent the vertical and horizontal movement of pollutant (Kabata-Pendias et al. 2010).

3.6.1.2 Separation of Heavy Metals Mechanically

This method involves separation of larger noncontaminated particles from smaller contaminated particles (Wuana and Okieimen 2011).

3.6.1.3 Remediation of Heavy Metals by Chemical Treatment

This technology involves use of chemicals such as hydrogen peroxide and chlorine to reduce the heavy metals movement in situ. This technology is performed in situ and has disadvantage of causing new source of pollution (Kabata-Pendias et al. 2010).

3.6.1.4 Electroremediation

This method involves passing of current having low intensity between anode and cathode in heavy metal polluted soil. In this process, metals can be recovered or removed through precipitation and electroplating (Kabata-Pendias et al. 2010).

3.6.1.5 Binding of Chemicals with Different Chelating Agent

This technique involves use of chemicals that may be organic and inorganic as chelating agent to bind the heavy metals. This process takes place in reactors. The chemicals involved in this process are organic acids and EDTA. The cleaned soil from which metals are removed is then returned to its former location. The efficacy of this process depends on the characteristics of soil (Kabata-Pendias et al. 2010).

3.6.1.6 Removal of Metals by Ion-Exchanging Process

This technique involves use of ion-exchanging materials to remove metal from contaminated soil. Ion-exchanging materials used in this process are chelating resins, zeolites, plant, and microbial biomass. This technique depends on pH and disadvantage of this technique is high cost (Kabata-Pendias et al. 2010).

3.6.2 Remediation of Metals by Biochemical Methods

Biochemical methods of metal remediation are as follows.

3.6.2.1 Bioleaching

This technique involves use of living organisms to extract the heavy metals from their ores. This technique uses several sulfur- and iron-oxidizing bacteria such as *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. These species are responsible for the formation of sulfuric acid from the oxidation of inorganic sulfur. This acid acts as metal chelator and used to remove the heavy metals from contaminated soil. *Aspergillus niger* is also involved in bioleaching process (Mulligan et al. 2004).

3.6.2.2 Biosorption

Biosorption involves concentration and interaction of organic pollutants or toxic metals in the biomass; this is taken as a potential tool for the remediation of metal-contaminated sites and for recovery of costly metals, offering a substitute to old methods such as adsorption and ion exchange on activated carbon. In biosorption, pollutants are bound to bacterial cell wall surface and are used to remove heavy metals from wastewaters, ground waters, and contaminated soils (Chojnacka 2010; Ansari and Malik 2007).

Bioremediation is an in situ remediation technique providing more advantages over the conventional chemical and physical treatments (Radhika et al. 2006).

3.6.2.3 Metal–Microbe Interactions

Metal–microbe interactions are of utmost importance both from plant growth promotion and bioremediation point of view (Ianeva 2009). Although certain heavy metals are needed by the plants for metabolic functioning but their higher concentrations are toxic to the plants (Hynninen et al. 2009). On the contrary, nonessential heavy metals are poisonous to plants and animals. Such metals are arsenic, cadmium, lead, and mercury. They are therefore termed as "toxic metals" (Janssen et al. 2010). These enter into the plant body through the uptake system of essential nutrient elements. At molecular level, these heavy metals get attached with thiol groups and did not allow essential metals to attach. Basically these nontoxic metals displace the essential metals (Ca, K, and Mg) and attach themselves.

Moreover, Pb has the ability to enter into plant body by attaching with the Ca and Zn transport proteins. Resultantly, conformational arrangements of proteins, enzymes, and nucleic acids are disrupted. It also causes disturbance in membrane functions, osmotic balance, and interference with oxidative phosphorylation (Bruins et al. 2000). Bioavailability of metals/metalloids governs their toxicity to the microorganisms. Consequently, with the decrease in pH, the bioavailability of metals to plants increases. It is because of more available concentration of metals in solution form. To overcome this situation, bacteria have adopted mechanisms to resist the higher concentration of these metals. They either pump metals out of their body or hyperaccumulate by converting into less toxic form (Bruins et al. 2000; Ianeva 2009). Additionally, bacterial population exists in the environments with high metal concentration (Bruins et al. 2000). Under heavy metal-stressed environment, microbes have developed numerous mechanisms to help them out. They can mobilize, immobilize, or transform metals. It renders metal ions subjected to plant intake (Shukla et al. 2010). Microorganisms in metal contamination can use either single or combination of mechanisms for existence (Hu et al. 2006).

Isolation of bacterial strains, resilient to the heavy metal toxicity, has been reported in many studies. Since 1970s, aerobic bacteria were mostly found resistant to the heavy metal infestation. Major examples of resistive microorganisms include species of *Bacillus* and *Staphylococcus*, in addition to *Pseudomonas aeruginosa* and *Escherichia coli* (Bruins et al. 2000). For instance, certain bacteria were identified to survive in Lead (Pb) and Zinc (Zn) mining sites even at massively high 204 μg Pb/g soil (Hu et al. 2006). Resilience to the heavy metal toxicity in bacteria may be due to genetic determinants localized on chromosomes and extra-chromosomal genetic materials like transposons and plasmids (Bruins et al. 2000). Transfer of genetic tendencies to the bacteria has a significant contribution in the heavy metal resistance (Gadd 2010). Among the various mechanisms involved in metal stress tolerance in bacteria (Shukla et al. 2010), the following five are of prime importance:

- Metal ion efflux
- Metal exclusion
- Enzymatic detoxification
- Intracellular sequestration
- Extracellular sequestration

Primarily, prokaryotes depict resistance to the toxicity by the active efflux of poisonous metal ions from the cell (Hynninen et al. 2009). Metal resistance system is majorly managed by this active efflux of ions (Bruins et al. 2000). However, intracellular complex formation (mainly in eukaryotes), certain binding factors, and enzyme-mediated reactions such as methylation, demethylation, oxidation, and reduction of metal also contribute significantly in preventing of adverse impact of heavy metals (Hynninen et al. 2009).

3.7 Metal Resistance Mechanisms Used by Microorganisms

Various genera of gram positive and negative bacteria have been identified for metal resilience in polluted soils (Taghavi et al. 2009b). Although in the current literature the mechanism of resistance to metal is still unknown and bacterial approaches of defense against metal toxicity are little known, some important strategies have been identified. In particular, mechanisms of active efflux and precipitation of the heavy metals in insoluble forms are common strategies employed by bacteria against heavy metals tolerance (Mire et al. 2004). Indeed energy-consuming transportation of ions against the gradient by employing ATP-dependent efflux pumps and sequestration of metal ions at the intracellular spaces are effective to remediate highly bioavailable metals accessing the cell membranes.

3.8 Metal Sequestration

Heavy metal-tolerant bacteria have the ability to sequester metals intra as well as extracellular and results in reduced mobilization of the metals. These bacteria actually use intra and extracellular mechanisms to avoid toxicity and it is well reported in the literature. A vast variety of these bacteria can precipitate metals particularly as metal–phosphate and some other forms. Accumulation of sequestered metal depends upon the type of bacterial strain, its growth stage, and environmental conditions containing metals (Mire et al. 2004).

3.8.1 Intracellular Sequestration

Intracellular sequestration is a metal accumulation strategy of bacteria in which metals are accumulated internally especially in cytoplasm to protect the other essential parts of the cell from exposure to toxic metals (Bruins et al. 2000; Mire et al. 2004). These bacteria have the ability to overexpress the Metallothionein (MT) genes after the exposure to heavy metals. MTs are cysteine-containing proteins with low molecular weight. These have high affinity for toxic (Cr and As) and essential

(Mn and Fe) metals. Abundant quantity of this protein is also present in fungi, animals, and plants. Proper biological function of MTs is still not illustrated properly but its role for metal sequestration is well documented. In fact the transcription of MT gene is induced by the metal such as Pb, as indicated for a *Streptomyces* strain's ability to resist to high concentration of the heavy metals such as Zn, Cu, and Pb (Rifaat et al. 2009). Intracellular sequestrations by binding proteins have also been reported in a *Synechococcus* sp. by producing MT proteins as a form of resistance (Bruins et al. 2000). Besides, the molecular mechanism remains to be elucidated, resistant strains of *Bacillus megaterium*, *Staphylococcus aureus*, *Citrobacter freundii*, and *Vibrio harveyi* have been reported to lower the concentration of free lead ions by precipitating lead and accumulating the metal as an intracellular cytoplasmic phosphate salt. In particular, the *Vibrio harveyi* strain was capable of precipitating Pb in large quantity as phosphate compound (Mire et al. 2004). The product accumulated by the *Citrobacter* sp. is recognized as $PbHPO_4$ and same precipitate also produced by *Staphylococcus aureus* strains. *Staphylococcus aureus* strains, both Pb-resistant and Pb-sensitive strains were able to sequester the lead, but only the Pb-resistant bacteria stored the metal as intracellular lead–phosphate in electron-dense inclusions. Actually metal sequestration by bacteria is a two-step process. In the first step, metals are attached to the negatively charged surface of the microbe. Negative charge on the surface of microbe is due to the negatively charged functional groups on the surface. In the second step, these metals are taken inside the body of microbe. Although Pb-sensitive cells also bind Pb(II) initially, the crystals of Pb–phosphate were not present in different compartments of the cells of sensitive isolates of bacteria as probably lacking the system for precipitating the metal as Pb–phosphate. After examination, negligibly soluble nontoxic phosphate crystals were found and the mechanism was supposed to continue until the Pb(II) concentration overwhelms the binding capacity of the cell (Levinson et al. 1996).

Metal-solubilizing bacteria precipitation has also been reported in a *Klebsiella* strain cultured in phosphate-limited medium. This bacterium was in fact able to precipitate $PbHPO_4$ granules on the cellular surface as reported for a *Citrobacter* species grown in the presence of lead, while it accumulates PBS in electron-dense granules in the cells in phosphate-limited cultures (Mire et al. 2004).

3.8.2 Extracellular Sequestration

Metal resistance based on extracellular sequestration results from the binding of toxic metal in a complex, thus it cannot enter the cell membrane. This mechanism has been found in bacteria and even in many species of yeast and fungi. *Saccharomyces cerevisiae* excretes large amounts of glutathione which may reduce absorption of Ni(II), which binds with great affinity to heavy metals. Other organisms such as yeast form insoluble complexes of phosphate to increase resistance (Bruins et al. 2000).

A lead-resistant *Pseudomonas marginalis* strain has been reported to avoid lead toxicity by precipitating it as an extracellular polymer. In the absence of Pb, *P. marginalis* still produced the polymer indicating that it is a metal independent process. *Extracellular* polymer production is a frequent and unique process by some microbes to overcome metal stress. Detailed and comprehensive investigations of microbial sequestered compounds are scarce. *P. fluorescens* produced precipitates that contain abundant phosphate and Pb. Furthermore, both phosphate-starved and phosphate-replete *P. fluorescens* cultures have been reported to generate an insoluble material containing both lead and phosphorus, although phosphate-replete cultures are apparently more efficient at expelling the material.

3.8.3 Plant–Microbe Interactions

Plant root surface and soil area around root called rhizosphere is a very complex medium that contains huge microbial activity. Rhizosphere contains about one to two fold more microbial population as compared to the bulk soil (Maier et al. 2009). It might be due to the high concentration of nutrients in the rhizosphere. As we move from rhizosphere to bulk soil, the nutrient concentration reduces and similar trend is observed in microbial population density. Furthermore, plant roots also produce organic metabolites that act as carbon, energy, and food source for the bacteria. Roots aerate rhizosphere to support the microbial activity (Belimov et al. 2001). Some of the bacteria in reverse have also the ability to support the plant either by enhancing the nutrient availability or by producing the plant growth regulators and protecting plant from the pathogens. This group of bacteria is called plant growth promoting rhizobacteria (PGPR). These bacteria consolidate the plant defense mechanisms under stress conditions like heavy metal stress, salinity, and drought (Erturk et al. 2010; Khan et al. 2009; Jing et al. 2007) and ultimately improve the plant growth in heavy metal-contaminated soil (Dary et al. 2010).

3.9 Phytoremediation of Contaminated Soils

It is a process that uses different plants to remediate the metal-contaminated sites either by extracting them out or stabilizing them in the soil. Some of the plants have the capacity to permanently remove the metals from soil by accumulating metal in under and above grounds parts while others produce such rhizospheric compounds that made compounds with metals by reducing their availability to the plants. In this process, metals remain in soil and only their mobility is minimized. The main process involved in the uptake of metals is absorption. This method is among the most economical and eco-friendly approach (Mangkoedihardjo, 2007).

Different processes involved in phytoremediation of heavy metal-contaminated soil are phytoextraction, phytostabilization, phytovolatilization, and microbe-assisted

phytoremediation. The process in which contaminants are contained by plant or immobilized in the soil or ground water is known as phytostabilization. It comprises the application of plants to decrease the bioavailability and movement of contaminants in soil. Plants directly stabilize contaminants by adsorption of the contaminants on the root surface, accumulation by the roots, or isolation within the root zone using plants as organic pumps (Pilon-Smits 2005). Phytovolatilization is the movement of a contaminant out of the soil or groundwater and into, through, and out of a plant into the atmosphere. In this process, the contaminant or its metabolite is released into the atmosphere (Pilon-Smits 2005). Phytoextraction (or phytoaccumulation) is the use of plants to remove pollutants from contaminated soil into their above ground parts which can then be harvested and it has been considered as a cost-effective, environment friendly strategy for the cleanup of metal-enriched soils (Manousaki and Nicolas 2009). Actually at the time of plant disposal, which can be composted or incinerated, contaminants are stored in the much smaller plant matter volume than in initially polluted soil or sediments. In fact, plants absorb heavy metals by the root system and concentrate them in the biomass of root and/or transport them into shoots and/or leaves, and plant may continue to uptake these heavy metals until it is harvested. After harvest, a minute concentration of heavy metals will remain in soil, so growth or harvest cycle must be repeated through many crops to get a significant cleanup. After this process, the soil can support other vegetations (Shukla et al. 2010).

However, higher contents of toxic metals in soil and water have resulted in more uptakes by roots and more translocation toward shoots, causing interference in normal metabolism and reduced growth. The success of phytoremediation is limited even with hyperaccumulators due to slow growth and less biomass production because of toxicity and elevated levels of metal ions. Phytoremediation alone is a time-consuming process and its success depends upon the metal tolerance, accumulation, and high biomass production capability of plants (Grčman et al. 2001). So, this situation could be improved and enhanced by assistance of plants with metal-tolerant bacteria having plant growth promotion activities (Ma et al. 2011; Khan et al. 2013).

3.10 Plant Growth-Promoting Rhizobacteria

Heterogeneous group of bacteria that have ability to enhance plant growth in association with plant roots inhabiting around the root is called plant growth-promoting rhizobacteria (PGPR). Mainly reported PGPR species are *Pseudomonas, Azotobacter, Klebsiella, Enterobacter, Arthrobacter, Azospirillum, Burkholderia, Serratia*, and *Bacillus*. In the past few years, comprehensive research work has been carried out to get the better understanding of mechanisms of PGPR (Khan et al. 2009). These bacteria can enhance the plant growth by different direct and indirect mechanisms. Direct mechanisms include nutrient availability (solubilization of mineral phosphates and nitrogen fixation and synthesis of siderophore) and production of plant growth regulators (indole-3-acetic acid (IAA), cytokinins, ethylene,

and gibberellic acid). While in indirect mechanism bacteria protect plant from pathogens by producing cyanide and antibiotics. But still exact mechanisms of plant growth promotion are not fully figured out (Erturk et al. 2010; Banerjee et al. 2010).

These bacteria are also helpful for the plant under stressed conditions. Under stressed conditions plants produce more ethylene that has a negative impact on plant growth. PGPR has the ability to reduce the plant ethylene level through enzymatic breakdown of ACC into ammonia and α-ketobutyrate. It is reported that establishment of ACC sink by bacterial population and reduction in the ethylene level consequently cause elongation of root, encourage the formation of longer roots, and decrease hazardous effects of stress that may increase the plant growth and seedling viability. Moreover, rhizobacteria play crucial role in the plant–bacterial interactions through the production of indole-3-acetic acid and phytostimulation efficiency. Under contaminated condition, biosynthesis of auxins and their release into the soil makes important contribution in plant growth promotion (Erturk et al. 2010). It is well documented that under high level of heavy metals condition, even metal-accumulating and tolerant plants are also affected by the heavy metals. So iron deficiency was detected in different plant species in the soil contaminated with heavy metals. Consequently, plant becomes chlorotic due to iron deficiency that causes inhibition of chloroplast development and chlorophyll biosynthesis. However, siderophores–iron complexes can mitigate the iron deficiency and act as a source of iron for plant. Under iron limiting conditions, siderophores produced by bacteria have iron acquisition ability in the form of Fe(III) chelators which is taken up by the plant roots (Kuffner et al. 2008).

3.11 Microbial-Induced Bioremediation

Microbial-induced bioremediation exploits the genetic and biochemical capacities of bacteria for the remediation of organic compounds and heavy metals. Therefore, due to the ability to tolerate metal toxicity, adsorb and accumulate heavy metals ions, or degrade organic pollutants, specific microorganisms can be studied and used in bioremediation of polluted environments. First, it is important to consider that every remediation approach is site specific and has to take into account the peculiar characteristics of the contamination and contaminated area. Moreover, no organisms or groups of organisms are universally applicable to all cases, although some can be metabolically versatile and are capable of degrading a wide spectrum of substrates, thus all procedures will be necessarily site specific. Depending on the detection in the contaminated matrix of metabolic activity functional to the contaminant detoxification, microbe induced-bioremediation relies on two approaches: biostimulation, stimulating native microbial population; and bioaugmentation, which imply an introduction of viable population to the contaminated area (Shukla et al. 2010). Actually if a functional metabolic activity is present, in a biostimulation protocol, soil conditions are modified to enhance catalytic capacities of autochthonous microorganism by supplementing nutrients (nitrogen and phosphorus) and/or electron acceptors (oxygen) until a decontaminated desired threshold is reached.

On the other side, in absence of a sufficient metabolic activity, functional to the contaminant remediation, it is possible to introduce a viable population with desired catalytic capabilities adopting a bioaugmentation protocol. In the case, a massive quantity of autochthonous microorganisms previously cultivated or allochthonous microorganisms with desired metabolic characteristics are bioaugmentated to the soil itself (Shukla et al. 2010). Bacteria can adopt bioaccumulation and biosorption mechanism. Bioaccumulation and biosorption involve concentration and interactions of organic pollutants or toxic metals in the biomass, either nonliving (biosorption) or living (bioaccumulation) is taken as a potential tool for the remediation of metal-contaminated sites and for recovery of costly metals, offering a substitute to old methods like adsorption and ion exchange on activated carbon. In biosorption, pollutants are bound to bacterial cell wall surface while in bioaccumulation these become accumulated under the cell. These both techniques are used to remove heavy metals from wastewaters, ground waters, and contaminated soils (Chojnacka 2010).

3.12 Plant Growth-Promoting Rhizobacteria-Assisted Phytoremediation

Phytoremediation can be considered as the most successful methodology for remediation of pollutants from contaminated water and soils. In this method, plant endurance and accumulation ability are very imperative (Paz-Alberto and Sigua 2013). Hyperaccumulators have the capability to extract considerable amounts of pollutants from shallow soil surfaces and water (Garbisu and Alkorta 2003). Different crops such as Indian mustard, sunflower, and alfalfa are efficient hyperaccumulators of Pb from soils but even then these gains are small size. In such a scenario, even by the use of hyperaccumulating plant for the removal of metals it could take years to completely remediate soils.

An alternative strategy to increase the efficiency of the phytoremediation is the inoculation with plant growth-promoting bacteria that facilitate the growth of hyperaccumulator in metal stress. In stress conditions, growth suppressing ethylene in plants can be lowered by inoculation with selected bacteria (Ahmad et al. 2011); such bacteria can also provide the plant with growth regulators and ultimately could improve the efficiency of phytoremediation (Fassler et al. 2010). Numerous findings have been reported, which support application of plant growth-promoting bacteria to facilitate metal phytoextraction (Table 3.1).

In stress conditions, microbial activity is also reduced (Asghar et al. 2012) but plants may help microbes by producing root exudates. Therefore, plant–microbes interaction can improve the phytoremediation efficiency. Phytoremediation assisted by soil rhizobacteria (also called rhizodegradation, rhizoremediation, enhanced rhizosphere biodegradation, microbially assisted phytoremediation) involves the breakdown of contaminants by mutual interaction of plant roots and microbes in the rhizosphere (Shukla et al. 2010). Plant root exudates act as source of carbon, energy, and nutrients for the microflora of soil and promote the activity of microbes (Shukla et al. 2010).

Table 3.1 PGPR-assisted phytoremediation

PGPR	Plant(s)	Metal(s)	Mechanism	References
Enterobacter sp. K3-2	*Sorghum sudanense*	Cu	Promote plant shoot and root growth, Phytostabilization of Cu, production of IAA, siderophore, ACC-deaminase, Arginine decarboxylase	Li et al. (2016)
Serratia sp. RSC-14	*Solanum nigrum*	Cd	Increase plant biomass and chlorophyll contents, improve phytoextraction of Cd	Khan et al. (2015)
Bacillus sp. RJ16	Tomato	Pb	Extensive rooting and reduced metal uptake Production of IAA, ACC-deaminase activity protecting tomato from growth inhibition	He et al. (2009)
Streptomyces tendae F4	Sunflower	Cd	Reduce metal accumulation by increasing iron content, increase siderophore secretion	Dimpka et al. (2009)
Rahnella aquatilis	Indian mustard	Cr	Promote biomass and rooting and reduce the uptake of cadmium; Increase siderophores, IAA production, and solubilization of inorganic phosphate	Kumar et al. (2009)
P. aeruginosa MKRh3	Lentil	Cd	Increase root and shoot biomass and reduce uptake of Cd Enhance siderophores, IAA, phosphate solubilization, and ACC-deaminase production	Ganesan (2008)
Pseudomonas sp.	Chickpea	Ni	Reduce translocation of Cd, enhance plant growth via production of siderophores, IAA, phosphate solubilization, and ACC-deaminase	Kuffner et al. (2008)
Enterobacter sp. NBRI K28	Indian mustard	Zn	Enhance root and shoot growth (height and weight) Reduce uptake of Cd Promote siderophores, IAA, phosphate solubilization and ACC-deaminase activity	Kumar et al. (2008)
Pseudomonas fluorescens	Sunflower	As	Plant growth promotion (mechanism unknown)	Shilev et al. (2006)
Psuedomonas aspleni AC	Canola	Cu	Enhance plant biomass and IAA production	Reed and Glick (2005)

Rhizospheric bacteria have ability to detoxify a variety of toxic metals/compounds efficiently. Different studies on rhizoremediation to explore the symbiotic association reported exhaustion of volatile organic contaminants, naphthalene and polychlorinated biphenyls and trichloroethylene (Shukla et al. 2010). Relationship between plant growth-promoting rhizobacteria and plant to enhance the uptake of toxic metals has been well established and recently phytoremediation associated with microbes has arisen as a successful strategy (Koo and Cho 2009).

PGPR have ability to enhance the growth of the host plant by various mechanisms involving production of specific compounds and increasing nutrient uptake. Further these bacteria can reduce the toxicity of heavy metals and promote the growth of plants under the toxicity of Ni, Pb, or As (Jing et al. 2007). Furthermore, some rhizobacteria can excrete organic acids to enhance the bioavailability of heavy metals and a variety of bacteria (mainly PGPR) have been reported as phytoextraction assistants, such as *Pseudomonas* spp., *Bacillus* spp., *Mesorhizobium* sp., *Microbacterium* spp., *Rhizobium* spp., *Sinorhizobium* sp., and *Achromobacter* sp. (Koo and Cho 2009).

Plant roots can also increase metal bioavailability by exuding low molecular weight organic acids and protons that cause decrease in pH of the soil and mobilize the metals. Retardation of heavy metals adsorption and high accumulation is just because of the decrease in the soil pH; moreover, the formation of soluble complex of heavy metals reacting with exuded organic acids also increases bioavailability of heavy metals to plants (Glick et al. 2007).

There are several other mechanisms and bacterial traits that increase the metals phytoremediation along with other previously reported growth promotion activities. For example, increased bioavailability of some metals for phytoremediation is supplemented with metal-binding peptides synthesis by genetically engineered bacteria (Wu et al. 2006). In addition, several scientists have found that efficient phosphate solubilization system present in bacteria can facilitate phytoremediation by its vital role in acquisition of metals. Metals bioavailability is increased when biosurfactant-producing bacteria are used in phytoremediation. The advanced experimentations on PGPR for the remediation of contaminated soils show a novel and innovative prospect for the successive studies. For example, rhizobacteria have proved to increase the acquisition of Cd in *Brassica napus* (Sheng and Xia 2006), of Ni in *Alyssum murale* (Abou-Shanab et al. 2007), and significantly improved Cu uptake by *B. juncea* (Ma et al. 2009). Along with free-living microbial association with plants to mitigate phytoremediation process, symbiotic relationship between metal-resistant rhizobial strains and their respective host has also promising output as metal uptake up to 80 % more in inoculated *M. pudica* than noninoculated plant has been observed (Chen et al. 2008).

3.13 Conclusions

Bacterial-assisted phytoremediation is considered a promising approach as compared to conventional remediation techniques for metal-contaminated soils. Plant growth-promoting rhizobacteria produce certain plant growth regulators and different enzymes that enhance plant growth under stress conditions. While in response plants provide carbon, energy, and nutrients in the form of root exudates and make conducive environment for the microbes.

However, there are several knowledge barriers that need to be addressed. Prominent among them include the understanding of the ecology and dynamics of PGPR under field conditions. Further, research needs to be focused on understanding the mechanism involved in the remediation process and their genetic characteristics.

References

Abou-Shanab R, Berkum PV, Angle JS (2007) Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of *Alyssum murale*. Chemosphere 68:360–367

Ahmad M, Zahir ZA, Asghar HN (2011) Inducing salt tolerance in mugbean through co-inoculation with Rhizobium and PGPR containing ACC-deaminase. Can J Microbiol 57:578–589

Ansari MI, Malik A (2007) Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater. Bioresour Technol 98:3149–3153

Asghar HN, Setia R, Marschner P (2012) Community composition and activity of microbes from saline soils and non-saline soils respond similarly to changes in salinity. Soil Biol Biochem 47:175–178

Banerjee S, Rakhi P, Chandan S, Standing D (2010) Stress induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. isolated from tomato rhizosphere. Aust J Crop Sci 4(6):378–383

Belimov A, Safronova V, Sergeyeva T, Egorova T, Matveyeva V, Tsyganov V, Borisov A, Tikhonovich I, Kluge C, Preisfeld A, Dietz K, Stepanok V (2001) Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocycloprop ane-1-carboxylate deaminase. Can J Microbiol 49(2):151–156

Binggan W, Yang L (2010) A review of heavy metal contaminations in urban soils, urban raod dusts and agricultural soils from China. Microchem J 94:99–107

Bruins MR, Sanjay K, Frederik O (2000) Microbial resistance to metals in the environment. Ecotoxicol Environ Saf 45(3):198–207

Chen WM, Wu CH, James EK, Chang JS (2008) Metal biosorption capability of *Cupriavidus taiwanensis* and its effects on heavy metal removal by nodulated *Mimosa pudica*. J Hazard Mater 151:364–371

Chojnacka K (2010) Biosorption and bioaccumulation-the prospect for practical applications. Environ Int 36:299–307

Dary M, Chamber-Pérez MA, Palomares AJ, Pajuelo E (2010) "In situ" phytostabilization of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater 177(1–3):323–330

Dimkpa CO, Merten D, Svatoš A, Büchel G, Kothe E (2009) Siderophores mediate reduced and increased uptake of cadmium by Streptomyces tendae F4 and sunflower (Helianthus annuus), respectively. J Appl Microbiol 107(5):1687–1696

Erturk Y, Ercisli S, Haznedar A, Cakmakci R (2010) Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. J Biol Res 43(1):91–98

Fassler E, Evangelou MW, Robinson BH, Schulin R (2010) Effects of indole-3-acetic acid (IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid (EDDS). Chemosphere 80:901–907

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 159:609–643

Ganesan V (2008) Rhizoremediation of cadmium soil using a cadmium-resistant plant growth-promoting rhizopseudomonad. Curr Microbiol 56(4):403–407

Garbisu C, Alkorta I (2003) Basic concepts on heavy metal soil bioremediation. Eur J Mineral Process Env Protect 3:229–236

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26(5-6):227–242

Gopal R, Khurana N (2011) Effect of heavy metal pollutants on sunflower. Afr J Plant Sci 5:531–536

Grčman H, Velikonja-Bolta Š, Vodnik D, Kos B, Leštan D (2001) EDTA enhanced heavy metal phytoextraction: metal accumulation leaching and toxicity. Plant Soil 235:105–114

He LY, Chen ZJ, Ren GD, Zhang YF, Qian M, Sheng XF (2009) Increased cadmium and lead uptake of a cadmium hyperaccumulator tomato by cadmium-resistant bacteria. Ecotoxicol Environ Saf 72(5):1343–1348

Hu Q, Hong-yan Q, Jing-hai Z, Hong-xun Z (2006) Bacterial diversity in soils around a lead and zinc mine. J Environ Sci 19(1):74–79

Hynninen A, Thierry T, Leena P, Dominique M, Marko V (2009) An efflux transporter PbrA and a phosphatase PbrB cooperate in a lead-resistance mechanism in bacteria. Mol Microbiol 74(2):384–394

Ianeva OD (2009) Mechanisms of bacteria resistance to heavy metals. Microbiology 71(6):54–65

Janssen PJ, Rob VH, Hugo M, Pieter M, Nicolas M, Mohammed B, Natalie L, Tatiana V, Alla L, Arlette M, Se'bastien M, Claudine T, Safiyh Sean M, John D (2010) The complete genome sequence of *Cupriavidus metallidurans* strain CH34, a master survivalist in harsh and anthropogenic environments. PLoS One 5(5):10433

Jing YZ, He Z, Yang X (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci 8:192–207

John R, Ahmad P, Gadgil K, Sharma S (2009) Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica junica* L. Int J Plant Prod 3:1735–8043

Kabata-Pendias A (2010) Trace elements in soils and plants. CRC, Boca Raton

Khan MS, Almas Z, Ahmad WP, Mohammad O (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19

Khan MY, Asghar HN, Jamshaid MU, Akhtar MJ, Zahir ZA (2013) Effect of microbial inoculation on wheat growth and phyto-stabilization of chromium contaminated soil. Pak J Bot 45(SI): 27–34

Khan AR, Ullah I, Khan AL, Park G, Waqas MS, Hong BK, Jung Y, Kwak Y, Lee I, Shin J (2015) Improvement in phytoremediation potential of Solanum nigrum under cadmium contamination through endophytic-assisted *Serratia* sp RSC-14 inoculation. Environ Sci Pollut Res 22:14032–14042

Koo S, Cho K (2009) Isolation and characterization of a plant growth-promoting *Rhizobacterium*, *Serratia* sp SY5. J Microbiol Biotechnol 19(11):1431–1438

Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A (2008) Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. Plant Soil 304:35–44

Kumar KV, Singh N, Behl HM, Srivastava S (2008) Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in Brassica juncea grown in fly ash amended soil. Chemosphere 72(4):678–683

Kumar KV, Srivastava S, Singh N, Behl HM (2009) Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of Brassica juncea. J Hazard Mater 170(1):51–57

Levinson HS, Mahler I, Blackwelder P, Hood T (1996) Lead resistance and sensitivity in *Staphylococcus aureus*. FEMS Microbiol Lett 145:421–425

Li Y, Wang Q, Wang L, He L, Sheng X (2016) Increased growth and root Cu accumulation of *Sorghum sudanense* by endophytic *Enterobacter* sp. K3-2: implications for *Sorghum sudanense* biomass production and phytostabilization. Ecotoxicol Environ Saf. doi:10.1016/j.ecoenv.2015.10.012

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258

Maier RM, Ian LP, Charles PG (2009) Environmental microbiology. Elsevier Inc, Amsterdam

Mangkoedihardjo S (2007) Phytotechnology integrity in environmental sanitation for sustainable development. J Appl Sci Res 3(10):1037–1044

Manousaki E, Nicolas N (2009) Phytoextraction of Pb and Cd by the Mediterranean saltbush (*Atriplex halimus* L.): metal uptake in relation to salinity. Environ Sci Pollut Res 16:844–854

Mire CE, Jeanette AT, William FO, Kandalam VR, Gregory BH (2004) Lead precipitation by *Vibrio harveyi*: evidence for novel Quorum-sensing interactions. Appl Environ Microbiol 70(2):855–864

Mohammad MJ, Malkawi HI, Shibli R (2003) Effects of mycorrhizal fungi and phosphorous fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. J Plant Nutr 26:125–137

Mulligan CN, Kamali M, Gibbs BF (2004) Bioleaching of heavy metals from a low-grade mining ore using *Aspergillus niger*. J Hazard Mater 110:77–84

Oancea S, Foca N, Airinei A (2005) Effects of heavy metals on plant growth and photosynthetic activity. Boil Trace Element Res 92:257–273
Paz-Alberto A, Sigua G (2013) Phytoremediation: a green technology to remove environmental pollutants. Am J Clim Chang 2:71–86
Peralta JR, De la Rosa G, Gonzalez JH, Torresdey JLG (2004) Effects of growth stages on the heavy metal tolerance of alfalfa plants. Adv Environ Res 8:679–685
Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39
Radhika V, Subramanian S, Natarajan KA (2006) Bioremediation of zinc using *Desulfotomaculum nigrificans*: Bioprecipitation and characterization studies. Water Res 40:3628–3636
Reed ML, Glick BR (2005) Growth of canola (Brassica napus) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can J Microbiol 51(12):1061–1069
Rifaat HM, Fathy MK, Wagdy KBK (2009) Effect of heavy metals upon metallothioneins in some *Streptomyces* species isolated from Egyptian soil. J Appl Sci Environ Sanit 4(3):197–206
Rouphael Y, Cardarelli M, Reab E, Colla G (2008) Grafting of cucumber as a means to minimize copper toxicity. Environ Exp Bot 63:49–58
Sanayei Y, Ismail N, Talebi SM (2009) Determination of heavy metals in Zayandeh Rood River, Isfahan-Iran. World Appl Sci J 6:1209–1214
Sheng XF, Xia JJ (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere 64:1036–1042
Shi Z, Tao S, Pan B, Fan W, He XC, Zuo Q, Wu SP, Li BG, Cao J, Liu WX, Xu FL, Wang XJ, Shen WRV, Wong PK (2005) Contamination of rivers in Tianjin, China by polycyclic aromatic hydrocarbons. Environ Pollut 134:97–111
Shilev S, Fernández A, Benlloch M, Sancho ED (2006) Sunflower growth and tolerance to arsenic is increased by the rhizospheric bacteria Pseudomonas fluorescens. In: Phytoremediation of metal-contaminated soils. Springer, Dordrecht, pp 315–318
Shukla KP, Singh N, Shivesh S (2010) Bioremediation: developments, current practices and perspectives. Genet Eng Biotechnol J GEBJ-3:1–20
Singh JS, Abhilash PC, Singh HB, Singh RP, Singh DP (2011) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480:1–9
Taghavi S, Celine L, Sebastien M, Ruddy W, Max M, Daniel VL (2009) Lead (II) resistance in Cupriavidus metallidurans CH34: interplay between plasmid and chromosomally-located functions. Antonie Van Leeuwenhoek 96(2):171–182
Talanavoa V, Titou AF, Boeva NP (2000) Effect of increasing concentrations of lead and cadmium on cucumber seedlings. Biol Plant 43(3):441–444
Tumuklu A, Yalcin MG, Sonmez M (2007) Detection of heavy metal concentrations in soil caused by Nigde City garbage dump. Polish J Environ Stud 16(4):651–658
Tuna AL, Brum B, Yokas I, Coban E (2002) The effects of heavy metals on pollen germination and pollen tube length in the tobacco plant. Turk J Biol 26:109–113
Weiqiang L, Khan MA, Shinjiro Y, Yujji K (2005) Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis Thaliana*. Plant Growth Regul 46:45–50
Wu CH, Wood TK, Mulchandani A, Chen W (2006) Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72(2):1129–1134
Wuana, RA; Okieimen, FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol. doi:http://dx.doi.org/10.5402/2011/402647

Chapter 4
Cyanoremediation: A Green-Clean Tool for Decontamination of Synthetic Pesticides from Agro- and Aquatic Ecosystems

Arun Kumar and Jay Shankar Singh

Abstract Immense use of synthetic chemicals in agriculture has deleterious effects on the environment even outside agro-ecosystem, microbial biodiversity, water bodies, and on life especially at the end of food chain, including humans. Therefore, there is a need to develop some viable and eco-friendly tools to remove these lethal chemicals from the environment. Bioremediation has been considered as a less-expensive alternative to physical and chemical means to decontaminate and degrade the pesticides from the contaminated sites. A number of microorganisms such as bacteria, fungi, actinomycetes, and cyanobacteria have been reported to degrade the pesticides. However, cyanobacteria (formally known as blue–green algae—BGA), the only known group of prokaryotes, capable of oxygenic photosynthesis and ubiquitous in distribution, have the remarkable ability to survive in harsh environments. Therefore, cyanobacteria could be a potential bioagent in degradation of noxious chemicals including pesticides. As a bioremediating agent, cyanobacteria have some advantages over other microbes in bioremediation, i.e., phototrophic nature makes them self-sufficient in growth, ability to fix nitrogen, and ease in biomass recovery. Some efficient and potential cyanobacterial genera such as *Anabaena, Leptolyngbya, Microcystis, Nostoc, Spirulina,* and *Synechocystis* have been found to tolerate and degrade various pesticides and herbicides. Biodegradation capabilities of cyanobacteria can be improved through genetic engineering, which can be exploited as cost-effective and eco-friendly remediation technology. This review focuses on the potential of cyanobacteria in the biodegradation of synthetic chemical residues from agro- and aquatic ecosystems.

Keywords Bioremediation • Cyanobacteria • Ecosystems • Insecticides • Synthetic Pesticides • Cell Immobilization

A. Kumar
Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Raibarelly Road, Lucknow, Uttar Pradesh 226025, India

J.S. Singh (✉)
Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@bbau.ac.in

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_4

4.1 Introduction

Synthetic pesticides are excessively applied in current agriculture practices to protect crops from various diseases and damages caused by fungi, insects, mites, and nematodes, to protect crops from abundant growth of weeds, and to control vectors responsible for certain diseases like malaria, dengue in human beings (Freedman 1995; Palanisami et al. 2009). These pesticides are known to be persistent in nature, causing toxicity and teratogenicity. They also cause deleterious side effects, not only in the cultivated soils where they are applied, but also can be accumulated into food crops; but also can be accumulated into food crops; and finally enter in food chain (El-Bestawy et al. 2007). In agro-ecosystems, they affect the growth of nontarget organisms such as beneficial microorganisms which play very crucial role in soil fertility and enhance plant growth (Araujo et al. 2003). Apart from this, they can enter into aquatic ecosystems by spraying, drifting, leaching, surface runoff, discharges from the pesticide manufacturing plants, and by accidental spills; this leads to the killings of fishes and aquatic invertebrates (Akhtar et al. 2009).

Bioremediation is an effective and eco-friendly approach for the decontamination of synthetic pesticides from agro- and aquatic ecosystems; it is a microorganism-mediated transformation or degradation of pollutants into nontoxic or less-toxic substances (Singh et al. 2011a, b, c). The application of various organisms like bacteria, actinomycetes, algae, methanotrophs (Singh and Gupta 2016), and cyanobacteria for efficient bioremediation of pesticide has been reported. Cyanobacteria are successively applied in wastewater treatment to remove nitrogen and phosphorus, textile dyes, and heavy metals (Palanisami et al. 2009; Singh et al. 2016). Cyanobacteria have been shown to be highly effective degraders of pesticides (Megharaj et al. 1994; Singh et al. 2011a, b, c).

Cyanobacteria, generally known as blue–green algae, are considered among the oldest photosynthetic organisms on planet Earth that existed since about 2.6–3.5 billion years ago (Hedges et al. 2001). They show diverse morphology including unicellular, filamentous, and colonial forms; benthic as well as planktonic (Whitton and Potts 2000; Burja et al. 2001). Cyanobacteria can flourish in a variety of habitats: from marine to freshwater and to terrestrial ecosystems; from arctic to Antarctica and to tropical deserts (Kulasooriya 2011). Some filamentous cyanobacteria have endowed with specialized cells known as heterocysts, known for the sites of nitrogen fixation (Capone et al. 2005).

This chapter gives us little information on synthetic pesticides and their fate and impact on agro- and aquatic ecosystems, but prime focus is on cyanobacteria-mediated bioremediation or cyanoremediation of synthetic pesticides and also focuses; how immobilization and genetically engineering enhance the capability to tolerate and degrade the synthetic pesticides.

4.2 Synthetic Pesticides

According to FAO (1989), "Pesticides are natural or synthetic substances or mixture of substances intended for preventing, destroying, or controlling any pest including vectors of human or animal diseases, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs, or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies." Nowadays, the term "pesticide" is generally applied for synthetic chemicals used to prevent crop loss from various insects, fungi, bacteria, and nematodes; to suppress excess growth of weeds and other substances used for storage and transportation of agricultural commodities.

4.2.1 Classification of Synthetic Pesticides *(Based on Zacharia 2011; EPA 2012; Ortiz-Hernández et al. 2013)*

Synthetic pesticides could be classified according to their toxicity, chemical group, environmental persistence, target organism, or other features (Tables 4.1 and 4.2). According to their chemical nature, pesticides are divided into following groups:

4.2.1.1 Organochlorines

Organochlorine pesticides are organic compounds with five or more chlorine atoms, and they are widely used as insecticides for the control of a wide range of insects. Organochlorine pesticides also show long persistence in the environment. These pesticides (mostly insecticides) disrupt nervous system, leading to convulsions and paralysis of the insect and its eventual death. DDT, lindane, endosulfan, aldrin, dieldrin, and chlordane are the commonly used organochlorine pesticides.

4.2.1.2 Organophosphorous

Organophosphorous pesticides possess a phosphate group as their basic structure; this is defined by Schrader's formula:

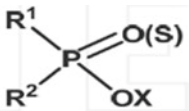


Table 4.1 Synthetic pesticides: types, mode of action and their examples

Types	Mode of action	Examples
Insecticides		
Organochlorines	Nervous system disruptors	Lindane, DDT, Heptachlor
Organophosphorous	Cholinesterase inhibitors, not specific	Malathion, Chloroprifos
Carbamates	Cholinesterase inhibitors, but specific	Carbendizm, Aldicarb
Pyrethroids	Synthetic analogues of the naturally occurring pyrethrins	Cypermethrin, Fenvalerate
Insect growth regulators	Inhibit endocrine or hormone system of insects	Azadirachtin, Methoprene
Nicotinic	Affect the central nervous system of insects	Imidacloprid, Acetamiprid
Pyrajole/Pyrrole	Inhibits mitochondrial electron transport	Chlorantrailiprole, Pyraclofos
Herbicides		
Phenoxics	Growth regulators	Bromofnoxim, 2,4,5-T
Trizines	Photosynthesis inhibitors(Photosystem II)	Trihydroxytrizine, Chlorazine
Benzoics	Growth regulators	Fenquinotrione
Sulfonylureas	ALS Inhibitors	Amidosulfuron
Bipyridilium	Photosynthesis inhibitors(Photosystem I)	Paraquat, diquat
Chloroacetamide	Shoot growth inhibitors	Acetochlor
Glycine	Aromatic amino acid synthesis inhibitors	Glyphosate
Dinitroaniline	Root growth inhibitors	Pendimethalin
Phenylpyrazoline	ACCase inhibitors	Fluazolate
Fungicides		
MBC	Inhibits tubulin formation in mitosis	Thiophanate-methyl
DMI	Sterol biosynthesis inhibition	Triforine, Tebuconazole
Phenylamide	Inhibits RNA synthesis	Mefenoxam
Anilopyrimidine	Methionine biosynthesis and hydrolytic enzymes	Cyprodinil, Pyrimethanil
QoI	Inhibits respiration (MET-III, cyto-bc1)	Azoxystrobin
Phenylpyrrole	Disrupts membrane integrity	Fludioxonil
Aromatic hydrocarbon	Thought to act on lipids	Dicloran, Etridiazole
Host plant defense inducers (SAR)	Activates plant's systemic acquired resistance (SAR)	Acibenzolar-*S*-methyl, Harpin

Note: *MBC* methyl benzimidazole carbamate; *DMI* demethylation inhibitor; *QoI* quinone outside inhibitor

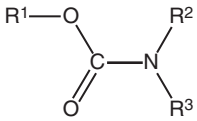
Table 4.2 Synthetic pesticides: classified according to their toxicity

Class	Toxicity
Ia	Extremely hazardous
Ib	Highly hazardous
II	Moderately hazardous
III	Slightly hazardous
IV	Product unlikely to present acute hazard in normal value

In this formula, R^1 and R^2 are usually methyl or ethyl groups; the O in the OX group can be replaced with S in some compounds, whereas the X group can take a wide diversity of forms. Organophosphorous insecticides are not persistent in the environment (Martin 1968) like organochlorine pesticides, but it is observed that they are more harmful for vertebrates and invertebrates due to cholinesterase inhibitors, leading to paralysis and death. Some of the widely used organophosphorous insecticides include parathion, malathion, diaznon, and glyphosate.

4.2.1.3 Carbamates

Carbamates are organic compounds which are derivatives of carbamic acid and defined through this formula:



Where R^1 is an alcohol group, R^2 is a methyl group, and R^3 is usually hydrogen. Carbamates (both aryl and oxime) are heavily toxic to insects and mammalians due to cholinesterase inhibitors. Although both carbamates and organophosphorous are cholinesterase inhibitors, the difference is in species specificity and reversibility (Drum 1980). Carbaryl, carbofuran, and aminocarb are the common example of carbamate pesticides.

4.2.1.4 Pyrethoids

Pyrethroids are synthetic equivalents of the naturally occurring pyrethrins extracted from flowers of *Chrysanthemum cinerariaefolium*. Pyrethroids are known to be very effective against insect pests, with minimal toxicity to mammals and easily biodegradable. The most widely used synthetic pyrethroids include permethrin, cypermethrin, and deltamethrin. Although less toxic and persistent than other groups of insecticides, they can still represent a problem. Pyrethroids display high

affinity to Na+-channels and its binding to these channels causes a prolonged channel opening that may result in a complete depolarization of the cell membrane thus blocking neuronal activity.

Other groups of synthetic pesticides that are widely used in control of weeds include among others phenoxyacetic acid under which the herbicide 2,4-D belongs and bipyridyls under which the herbicides paraquat and diquat belong.

There is another group that includes the pesticides which can be applied for the control of fungal infections in crops. There are inorganic and organic fungicides. Inorganic fungicides include Bordeaux mixture, $Cu(OH)_2.CaSO_4$ and malachite, $Cu(HO)_2{\cdot}CuCO_3$. Organic fungicides, on the other hand, include among others, benomyl and xine copper (Manahan 2001).

4.3 Fate of Synthetic Pesticides in Agro- and Aquatic Ecosystems

Synthetic pesticides are applied in agriculture through various ways like spraying, dusting or spreading. These pesticides are taken up by pests or crop plants that are converted into degradable products and bio accumulated into plant parts or animal tissues (Babu et al. 2003; Waliszewski et al. 2008). Some parts of the pesticides applied in agricultural fields are also removed upon crop harvesting. The remaining parts of the pesticides can be degraded through chemical reactions and microbial actions in the soil, can be mineralized through sorption onto soil organic matter and clay minerals, and can also be lost to atmosphere through volatilization. Some synthetic pesticides that are not degraded, immobilized, detoxified, or removed with the harvested crop are escaped from the applied sites. The major loss pathways of pesticides to the environment are volatilization into the atmosphere and aerial drift, runoff to surface water bodies in dissolved and particulate forms, and leaching into groundwater basins (Fig. 4.1). The fate and transfer pathways of pesticide applied to crop plants are complex, requiring some knowledge of their chemical properties, their transformations (breakdown), and the physical transport process. Transforms and transport are strongly influenced by site-specific conditions and management practices.

4.4 Impact of Pesticides

Synthetic pesticides help enhancing economic potential through increased production of agricultural commodities and prevention of vector-borne diseases (Igbedioh 1991; Forget 1993). This negative impact of pesticides is mainly due to the high toxicity, stable nature, less soluble active ingredients of pesticide.

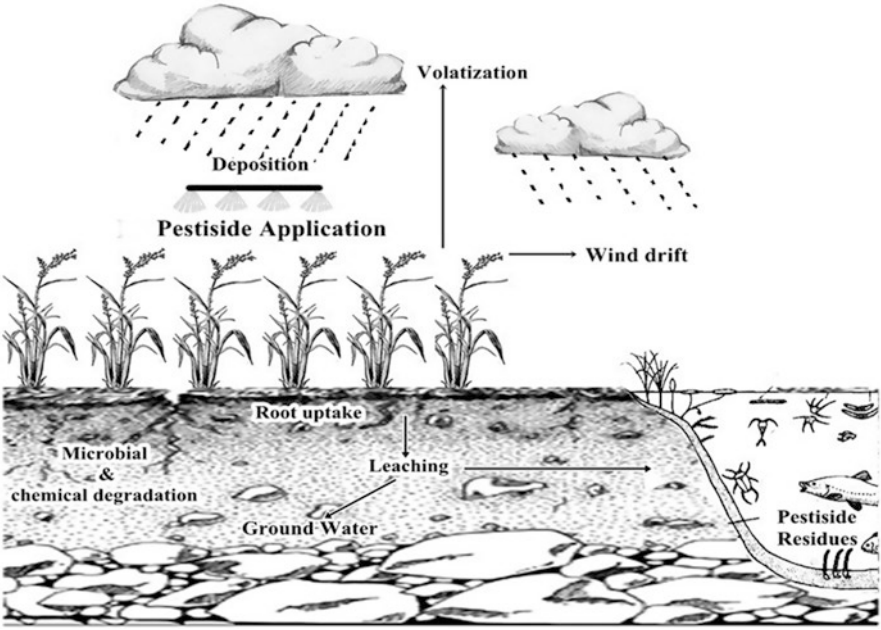


Fig. 4.1 Fate of synthetic pesticides in agro- and aquatic ecosystems

4.4.1 Soil Contamination

The major portion of the synthetic pesticides remains unused after application and is responsible for the contamination of the soil. In the soil, it can remain persistent, degraded, or transformed. Several researchers reported a variety of transformation products (TPs) from a wide range of pesticides (Barcelo and Hennion 1997; Roberts 1998; Roberts and Hutson 1999). The pesticides and their TPs are sorbed by soils to different degrees, depending on the interactions between soil and pesticide properties. The most influential soil characteristic is the organic matter content; larger the organic matter content, the greater the adsorption of pesticides and TPs (Akhtar et al. 2009).

4.4.2 Surface and Groundwater Contamination

From applied sites, pesticides can escape to surface water through runoff from treated plants and soil. Contamination of surface water by pesticides is a widespread problem. During a survey in India, 58 % of drinking water samples drawn from various hand pumps and wells around Bhopal were contaminated with organochlorine pesticides above the EPA standards (Kole and Bagchi 1995). Once ground water is polluted with toxic chemicals, it may take many years for the contamination to dissipate or be cleaned up. Cleanup may also be very costly and complex, if not impossible (Waskom 1994; O'Neil et al. 1998; US EPA 2001).

4.4.3 Effect on Soil Fertility

Due to indiscriminate use, pesticides have a negative impact on beneficial soil microorganisms. Elaine Ingham stated that if both bacteria and fungi populations are affected, then the soil starts to degrade. Overuse of chemical fertilizers and pesticides have the same side effects on the soil organisms that are similar to human overuse of antibiotics. Although after application of chemicals, it takes days, months, or years to be sort out or escape, but after a while, there aren't enough "beneficial soil organisms to hold onto the nutrients" (Savonen 1997). Soil microorganisms play a vital role in plants in terms of transformation of atmospheric nitrogen into nitrates, which plants can use, enhancing bioavailability on nutrients.

4.4.4 Nontarget Organisms

Nowadays, synthetic pesticides are found as common contaminants in soil, air, water, and our urban landscapes. They can also harm plants and animals ranging from nontarget insects, plants, fish, birds, and other wildlife. Synthetic pesticides are continuously applied and can be responsible for the extinction of useful organisms present in the agro- and aquatic ecosystems. Pesticide residues not only affect the soil features but also affect useful organisms like earthworms, bees, spiders, and plants (Singh et al. 2014).

4.4.5 Contamination of Vegetation

Pesticide application can directly affect nontarget vegetation or can drift or volatilize from the applied area and contaminate air, soil, and nontarget plants. Some pesticide drift occurs during every application, even from ground equipment (Glotfelty and Schomburg 1989). Pesticide drift can be responsible for a loss of 2–25 % of the pesticide being applied, which can spread over a distance of a few yards to several hundred miles and after few days of application, up to 80–90 % of an applied pesticide can be volatilized (Majewski and Capel 1995).

4.4.6 Human Health

Increase in the use of pesticides can result in various health and environmental problems like poisoning of farmers and farm workers, leading to cardiopulmonary, neurological, and skin disorders, fetal irregularities, miscarriages, lowering the sperm count of applicators, etc. These are categorized into acute and chronic poisoning: (a) Acute pesticide poisoning causes fatigue, headaches and body aches, skin discomfort, skin

rashes, poor concentration, feelings of weakness, circulatory problems, dizziness, nausea, vomiting, excessive sweating, impaired vision, tremors, panic attacks, cramps, etc., and in severe cases, coma and death (Bödeker and Dümmler 1993; Alavanja et al. 2004); (b) Chronic poisoning due to pesticide use or due to long-term ingestion of small amounts of these substances include weakening of the immune system and effects on the reproductive system, which can lead to miscarriage, still birth, and premature birth or to low birth weight(WWF 2002; UNEP 2004; Terre Des Hommes 2011).

4.5 Bioremediation of Synthetic Pesticides

Conventionally, bioremediation of synthetic pesticides is attained through the use of microorganisms; but nowadays, several other gents such as plants, fungi, algae, or enzymes (obtained from organisms) are also used in bioremediation which extends the application of bioremediation in various aspects. Bioremediation of synthetic pesticides includes two terms, biodegradation and biotransformation, recognized similar to each other but they are quite different.

Biodegradation involves the biological reactions that modify the chemical structure of the compound so this implies a decrease in toxicity, while biotransformation reduces the pollutant concentration by either modification or translocation. Thus, biotransformation could end decreasing or increasing the undesirable effects. Their difference is clear in the case of pollutants translocation when biodegradation does not occur but biotransformation does. Biotransformation concept has been developed for biological detoxification systems (Alexander 1999; Parkinson 2001). When microorganisms are imported to a contaminated site to enhance degradation, the process is known as bioaugmentation (Murali et al. 2014).

4.6 Factors Affecting the Bioremediation of Synthetic Pesticides

The biodegradation or biotransformation of synthetic pesticides is a complex process, and it is influenced by several physical and chemical attributes such as structure and concentration of pesticide, environmental conditions (temperature, pH, moisture), salinity, and sustainable population of microorganisms.

4.6.1 Structure and Concentration of Pesticide

The structure of synthetic pesticides is an important attribute; pesticides have some of their own physical and chemical properties which varyfrom pesticide to pesticide. Cork and Krueger 1991 stated that in a pesticide polar group such as OH, COOH and NH_2^{+3} are available to the microbial system; it could be an easier site for

attack but if the pesticide molecule is available as a substituent of halogen or alkyl, it makes it more resistant to biodegradation. The rate of degradation of pesticides can be influenced by minor difference in the arrangement or nature of substituent in pesticides of the same class (Topp et al. 1997). Beside the structure, the concentration of pesticide considerably affects the bioremediation of pesticides. The rate of degradation decreases generally quantitatively with the residual pesticide concentration (Topp et al. 1997).

4.6.2 Effect of Temperature, pH, and Moisture

Various environmental factors such as temperature, pH, and moisture also affect the process of biodegradation of synthetic pesticides. According to Alexander 1977, the entire process of biodegradation is carried out at mesophillic (30–37 °C with optimum temperature 35 °C) and thermophillic (50–60 °C with optimum temperature 55 °C) temperature ranges. The optimal temperature required for both the ranges is not invariably critical for the biodegradation.

Soil pH is a crucial factor for adsorption of pesticides for the abiotic and biotic degradation processes, and it also effects the adsorption behavior of pesticide molecules on clay and organic surfaces. This also affects the chemical speciation, mobility, and bioavailability (Burns 1975; Hicks et al. 1990). Racke et al. (1997) reported that degradation of a given pesticide depends mostly on the soil alkaline or acidic pH. In fact, the biodegradation of pesticides depends upon the susceptibility of the microorganism in the optimum pH of the medium. Moisture is another environmental factor which affects the rate of biodegradation; water facilitates as medium for the movement and diffusion of pesticides; it is necessary for microbial availability of pesticides.

Moisture maintains the osmotic pressure and pH of agro- and aquatic ecosystems; it also affects the exchange of respiratory gases in pore spaces of soil. Under saturated conditions, oxygen can be consumed faster than it is replenished in the soil space and the soil becomes anaerobic; this leads to slowing the rate of biodegradation and also changes metabolic activity of microorganisms to occur. Soil moisture content should be between 25 and 85 % of the water holding capacity (with optimum range of 50–80 %) for effective biodegradation of synthetic pesticides.

4.6.3 Effect of Salinity

There is not much information about the effects of salinity on the degradation of synthetic pesticides. Salinity is a big problem in many arid, semiarid, and coastal regions; it could affect the biodegradation of synthetic pesticides. Reddy and Sethunathan (1985) reported that parathion degradation is faster in nonsaline soils. It is also reported that the stability of pesticides in estuarine and sea water, varying

degrees of salinity; high salt content in seawater may be barrier for biodegradation (Walker 1976) or inhibit biodegradation of pesticides (Weber 1976; Kodama and Kuwatsuka 1980).

4.6.4 Sustainable Population of Microorganisms

Although microorganisms are able to survive in subzero temperatures, extreme heat, desert conditions, in aerobic or anaerobic conditions, with the presence of hazardous compounds but for the effective biodegradation of synthetic pesticides, it is necessary to meet these variables such as availability of pesticide or metabolite to the microorganisms, physiological status of the microorganisms, survival and/or proliferation of pesticide degrading microorganisms at contaminated site and most important is sustainable population of these microorganisms (Singh 2008).

4.7 Cyanoremediation

Cyanoremediation is the use of cyanobacteria for the removal or degradation or transformation of pollutants including heavy metal, dyes, or pesticides from wastewater or contaminated soil. Figure 4.2 illustrates the advantages using cyanobacteria over other microbes for bioremediation of pesticide contamination. There are numerous examples of cyanobacterial genera which are successfully implemented for the bioremediation of synthetic pesticides (Table 4.3).

According to Hatzios (1991), pesticide degradation is a process involving three phases: (a) Phase I involves oxidation, reduction, or hydrolysis, which makes the pesticide more water soluble and less toxic pesticide metabolites. In this phase, oxygenation is the crucial step in the degradation of pesticides and many of oxygenation reactions are carried out by oxidative enzymes, e.g., cytochrome P_{450}s, peroxidases, and polyphenol oxidases. (b) Phase II involves conjugation of a pesticide or pesticide metabolites to a sugar, amino acid or glutathione, which enhances the water solubility and reduces the toxicity compared to parent pesticide compound. Generally, metabolites obtained from Phase II have little or no toxicity and may be stored in cellular organelles. In this phase, enzyme Glutathione S-transferase plays a great role which catalyzes the nucleophilic attack of the sulfur atom of GSH by the electrophilic center of the substrate (Armstrong 1994; Marrs 1996); (c) Phase III involves conversion of Phase II metabolites into secondary conjugates, which are also nontoxic.

In the degradation process, pesticides produce singlet oxygen and other active oxygen species at various sites of photosynthetic electron transport chain. These active oxygen species are scavenged by cellular systems through raising antioxidative machinery such as superoxide dismutase, catalase, and peroxidase (Palanisami et al. 2009).

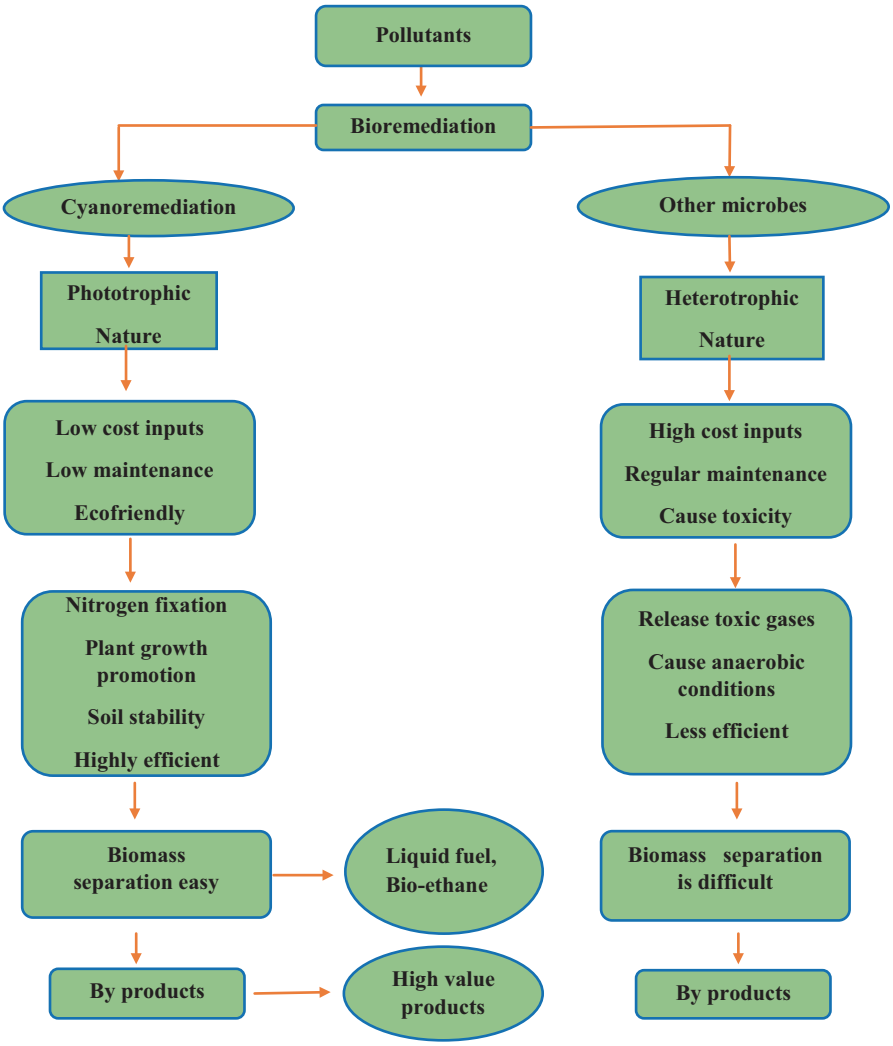


Fig. 4.2 Advantages of using Cyanobacteria over other microbes for bioremediation

4.7.1 Organochlorine Insecticides

Organochlorines are chlorinated hydrocarbon chemicals used to control various agricultural, horticultural, and public health pests (Lal et al. 2010). Their residues cause serious problems, not only in the cultivated soils where they are applied, but also in the crops that systematically retain part of these residues in nontarget organisms (El-Bestawy et al. 2007; González et al. 2012).

Table 4.3 Some synthetic pesticides and cyanobacteria species responsible for their degradation

Synthetic pesticides	Cyanobacteria	References
2,4-D (Dichlorophenoxyacetic acid)	*Anabaena fertilissima, Aulosira fertilissima, Westiellopsis prolifica*	Kumar et al. (2013)
2,4-DNP (Dinitrophenol)	*Anabaena variabilis* *A. cylindrica*	Hirooka et al. (2006)
Anilofos	*Synechocystis* sp. Strain PUPCCC 64	Singh et al. (2013)
Acetachlor	Cyanobacteria mat consisting *Phormidium and Oscillatoria*	El-Nahhal et al. (2013)
Carbaryl	*Calothrix berevissima*	Habib et al. (2011)
Carbendizm	*Oscillatoria* sp.	Ravindran et al. (2000)
Carbofuran	*Anabaena sphaerica, Nostoc hatei, Westiellopsis prolifica*	Jha and Mishra (2005)
Chlorpyrifos	*Phormidium valderianum, Spirulina platensis, Synechocystis* sp. *Strain PUPCCC64*	Palanisami et al. (2009)
Cypermethrin	*Oscillatoria*	Thengodkar and Sivakami (2010)
Endosulfan	*Anabaena* sp. PCC 7120 *A. flos-aquae* *Aulosira fertilissima*	Singh et al. (2011a, b, c) Ravindran et al. (2000) Lee et al. (2003)
Fenamiphos	*Nostoc muscorum, Anabaena* sp.	
Glyphosate	*S. platensis, N. punctiforme, M. aeroginosa, L. boryana*	Kumar et al. (2012); Caceres et al. (2008); Forlani et al. (2008); Lipok et al. (2009)
Isoproturon	*Anabaena inaequalis*	Arunakumara et al. (2013)
Lindane	*M. aeruginosa, Pseudoanabaena limnetica*	Mostafa and Helling (2001)
	Anabaena sp. *Strain PCC 7120* *Nostoc ellipsosporum*	González et al. (2012)
Malathion	*Anabaena oryzae, N. muscorum, S. platensis*	Kuritz and Wolk (1995)
	Anabaena sp. *Strain PCC 7120*	El-Bestawy et al. (2007)
Methyl parathion	*Anabaena fertilissima, Aulosira fertilissima, Westiellopsis prolifica*	Ibrahim et al. (2014)
Monocrotophos Penycuron		Barton et al. (2004) Kumar et al. (2013)

Among organochlorines, lindane (a common A-hexachlorocyclohexane (HCH) formulation) is a wisely applicable pesticide, mainly used for rice crop protection in rice-producing countries (Abdullah et al. 1997). Lindane persists in the environment (Alexander 1994) and can be noticed in the air, rain, and surface water at 90 % of sites long after its application (Majewski and Capel 1995). Singh (1973) reported that some cyanobacterial strains isolated from paddy fields, i.e., *Cylindrospermurn* sp., *Aulosira fertilissirna*, and *Plectonema boyanurn*, are able to tolerate commercial preparations of lindane in concentrations up to 80 pg/mL. Kuritz and Wolk (1995) also showed that two laboratory strains, *Anabaena* sp. PCC7120 and *Nostoc ellipsosporurn*, degraded A-HCH to a mixture of 1,2,3-and 1,2,4-trichlorobenzenes (and, possibly, beyond) via pentachlorocyclohexene as an intermediate. It is also observed that lindane did not affect the growth rates of these cyanobacteria at concentrations up to 20 pg/mL (Singh 1973).

It is reported that *Anabaena* sp. Strain PCC 7120 and *Anabaena flos-aquae* biotransformed endosulfan into endodiol, primary product and trace the amount of endosulfan sulfate (Lee et al. 2003). Endodiol is a nontoxic metabolite to fish and other organisms. But endosulfan sulfate has a similar toxicity compared to parent compound endosulfan, and it has a much longer tolerance into soil environment in comparison to endosulfan (Kennedy et al. 2001).

4.7.2 Organophosphorous Insecticides

Organophosphorous insecticides are esters of phosphoric acids and commonly known as organophosphates, which include aliphatic, phenyl, and heterocyclic derivatives and have one of the basic building blocks as a part of their much more complex chemical structure. They are applied for a variety of sucking, chewing, and boring insects, spiders and mites, aphids and pests attacking crops like cotton, sugarcane, peanuts, tobacco, vegetables, fruits, and ornamentals. Some of the main agricultural products are parathion, methyl parathion, chloropyrifos, malathion, monocrotofos, and dimethoate (Kanekar et al. 2004).

Organophosphorus pesticides are less environmentally persistent than organochlorine compounds; however, they still can be detected in air and water due to heavy use (Majewski and Capel 1995). In aquatic environments, nonenzymatic hydrolysis of organophosphates is responsible for their slow decomposition to more toxic and persistent para-nitrophenol (Megharaj et al. 1994). To overcome this problem, microalgae (including cyanobacteria)-mediated degradation could be an effective approach for their cleanup in the environment (Megharaj et al. 1994). Cyanobacteria are not so much affected by organophosphorus pesticides at working concentrations and concentrations present in wastewaters (Singh 1973; Doggett and Rhodes 1991; Megharaj et al. 1994; Subramanian et al. 1994). Pure cultures of *Nostoc*, *Oscillatoria*, and *Phomidium* isolated from methyl parathion-enriched soil, grew in media supplemented with methyl parathion or other organophosphorus pesticides as a sole source of organic phosphorus and nitrate (Megharaj et al. 1987; Orus and Marco 1991;

Megharaj et al. 1994; Subramanian et al. 1994) and utilized phosphorus from the pesticide for growth and development (Megharaj et al. 1994; Subramanian et al. 1994). Megharaj et al. (1994) stated that cyanobacteria are also able to oxidize the nitro group of para-nitrophenol accompanied by the release of nitrite into growth media, but enzymatic system which is involved in this process is not known. The metabolism/assimilation of the released nitrite is likely to depend on the activity of nitrite reductase encoded by the *nir operon*. Subramanian et al. (1994) also noted that the link between nitrogen metabolism and the effectiveness of phosphorus utilization from organophosphorus pesticides; however, the authors did not analyze possible effects of various sources of fixed nitrogen on biodegradation (Kuritz 1999).

Palanisami et al. (2009) reported that cyanobacterium *Phormidium valderianum* BDU 20041 tolerant to chloropyrifos exposure showed increased activity of oxidoreductase enzymes to degradation of chloropyrifos. *Sprirulina platensis* are able to grow in media containing up to 80 ppm choloropyrifos and converted to its primary metabolite TCP(3, 5, 6-trichloro-2-pyridinol) through the enzyme alkaline phosphatase (ALP) (Thengodkar and Sivakami 2010). Singh et al. (2011) concluded that cyanobacterium *Synechocystis* sp. Strain PUPCCC 64 is able to degrade the pesticide chloropyrifos. Three strains of cyanobacteria *Anabaena oryzae*, *Nostoc muscorum*, and *Spirulina platensis* are able to degrade and utilize malathion as a source of phosphorous. These strains grow under high concentration of malathion with enhancement of biomass carbohydrate and protein content (Ibrahim et al. 2014). It is also reported that cyanobacterium *Anabaena* sp. Strain PCC 7120 reduced the nitro group of methyl parathion to an amino group via a nitroso group intermediate under aerobic conditions (Barton et al. 2004).

4.7.3 Herbicides

Gimsing et al. (2004) reported that the degradation rate of pesticide is strongly correlated with the population size of soil microbes in case of *Pseudomonas* spp. Lipok et al. (2007) concluded that mixed culture of *Spirulina* sp. exhibited a remarkable ability to degrade glyphosate and the rate of glyphosate disappearance from the aqueous medium was independent of its initial concentration. They also suggested that the degradative pathway for glyphosate in *Spirulina* sp. might differ from those exhibited in other bacteria. In fact, Lipok et al. (2009) reconfirmed the ability of the cyanobacterium *S. platensis* and bacterium *Streptomyces lusitanus* to catalyze glyphosate metabolism. Four cyanobacterial strains (*Anabaena* sp., *L. boryana*, *M. aeruginosa*, and *N. punctiforme*) are able to use the glyphosate as the only source of phosphorus (Forlani et al. 2008). Dyhrman et al. (2006) also stated that marine cyanobacteria *Trichodesmium erythraeum* showed existence of phosphorous-dependent glyphosate transformation. However, reports on the utilization of glyphosate as a source of nitrogen by cyanobacteria are not yet available in the literature. Ravi and Balakumar (1998) reported that extracellular phosphatases are able to hydrolyze the C-P bond of glyphosate with working on cyanobacterium *A. variabilis*; however, this claim has not been

reiterated so far by the other authors. Forlani et al. (2008) stated that extracellular phosphatases seem unlikely to contribute any substantial scale to glyphosate degradation. Cyanobacterial strains which possess the ability to use this phosphonate as a source of phosphorus is of practically significance because such strains could effectively be employed for the cleanup of pesticides (Arunakumara et al. 2013).

4.8 Cyanobacterial Immobilization

The concept of immobilization of microorganisms in matrix or material may enhance the current benefits from the mass culture of the microorganism by degrading a specific metabolite or removing pollutants (De-Bashan and Yoav Bashan 2010; Ortiz-Hernández et al. 2011, 2013). And it can be employed for the bioremediation of synthetic pesticides because it confers the possibility of maintaining catalytic activity over long periods of time (Martín et al. 2000; Richins et al. 2000; Chen and Georgiou 2002). There are many advantages of immobilization of microorganisms over free-living microorganisms, such as the maintaining high cell density, the minimum cell washout, even at high dilution rates, easy separation of cells from the reaction system, repeated use of cells, and better protection of cells from the toxic effects of hazardous compounds and harsh environments. Immobilization can increase the cells' survival and metabolic activity in bioremediation systems (Moslemy et al. 2002; Tao et al. 2009; Ha et al. 2008, 2009; Sun et al. 2010). Two types of immobilization are as follows:

4.8.1 Passive Immobilization

Some microorganisms (including some groups of microalgae/cyanobacteria) have a natural tendency to attach to surfaces and grow on them (Robinson et al. 1986). This characteristic can be exploited in order to immobilize cells on carriers of different types (Codd 1987). In passive immobilization, carriers (adsorbent materials) can be natural or synthetic, and this process is reversible (Cohen 2001; Moreno-Garrido 2008). The natural carrier loofa biomass is widely used and accepted for passive immobilization while synthetic materials, polyvinyl and polyurethane, are widely used in experiments involving passive immobilization (Urrutia et al. 1995).

4.8.2 Active Immobilization

For active immobilization, a variety of carriers such as flocculant agents, chemical attachment, and gel entrapment are currently in use. Among flocculants, chitosan has been the most widely employed. Chemical attachment is carried out through the chemical interaction (mainly due to covalent bonding, cross-linking) by common

carriers such as glutaraldehyde, or cells. Apart from flocculant and chemical attachment, gel entrapment can be performed by the use of synthetic polymers (acrylamide, photocrosslinkable resins, polyurethanes), proteins (gelatine, collagen, or egg white), or natural polysaccharides (Taha et al. 2013).

Entrapment in natural polymeric gels has become the best suitable technique for the immobilization of cells (Mallick 2002); however, immobilized cells on supports have been used more frequently in xenobiotics biodegradation than for pesticides (Lusta et al. 1990). For cyanoremediation of synthetic pesticides, it is important to search for materials with favorable characteristics for the immobilization of cells, including aspects such as physical structure, ease of sterilization, and the possibility of using it repeatedly. Above all, the support must be affordable enough to allow its future use for pesticide degradation.

4.9 GE Cyanobacteria as Biopesticides

Gene manipulation offers a way of engineering microorganisms to deal with a pollutant, including pesticides that may be present in the contaminated sites. The simplest approach is to extend the degradative capabilities of existing metabolic pathways within an organism either by introducing additional enzymes from other organisms or by modifying the specificity of the catabolic genes already present. Cyanobacteria have long been studied as model organisms for photosynthesis (Vermaas 2001; Dong and Golden 2008); the engineering of cyanobacteria for applied purposes remains an underdeveloped field of interest. The potential of genetically modified cyanobacteria is still in the initial stages of exploration. Only a handful of cyanobacterial species have been investigated as host organisms for industrial and bioremediation purposes (Table 4.4). As new species are discovered and sequenced and new tools become available for genetic manipulation, the rich diversity of cyanobacterial phenotypes and genotypes can be exploited for new applications. Increased knowledge of native cyanobacterial genetics, metabolism, and regulatory systems will provide targets for increased production, enabling the synthesis of new products and improving the ability to predict the effects of targeted genetic manipulation.

Genetic engineering in filamentous N_2-fixing cyanobacteria usually involves *Anabaena* sp. PCC 7120 and several other non-aggregating species. Mass culture and harvest of such species are more energy consuming relative to aggregating species. To establish a gene transfer system for aggregating species, Qiong et al. (2010) tested many species of Anabaena and Nostoc and identified *Nostoc muscorum* FACHB244 as a species that can be genetically manipulated using conjugative gene transfer system. To promote biodegradation of organophosphorous pollutants in environments, they introduced a plasmid containing the organophosphorous degradation gene (opd) into *Anabaena* sp. PCC 7120 and *N. muscorum* FACHB244 by conjugation. The opd gene was driven by a story promoter, PpsbA. From both species, they obtained transgenic strains having organophosphorous degradation activities. The genetic manipulation of cyanobacteria could be utilized in the elimination of pollutants and large-scale production of valuable proteins or metabolites.

Table 4.4 Some GE cyanobacterial strains and their transformation methods

Cyanobacterial strains	Transformation methods	References
Anabaena & *Nostoc* sp. PCC 7120	Conjugation	Khasdan et al. (2003); Masukawa et al. (2007)
A.variabilis ATCC 29413	Conjugation	Roessler et al. (2009); Happe et al. (2000)
N. punctiforme ATCC 29133	Conjugation	Lindberg et al. 2002
Nostoc sp. PCC 7422	Conjugation	Yoshino et al. (2007)
Synechococcus elongatus PCC 7942	Natural	Niederholtmeyer et al. (2010); Kaczmarzyk and Fulda (2010);
	Natural	Takeshima et al. (1994)
Synechococcus sp. PCC 6301 *Synechococcus* sp. PCC 7002	Natural	McNeely et al. (2010); Reppas and Ridley (2010)
	Conjugation	Asada et al. (1999); Miyake et al. (2000)
Synechococcus sp. MA 19	Conjugation	Sode et al. (1998); Yu et al. (2000)
Synechococcus sp. NKBG15041c	Conjugation	Nobles and Brown (2008)
S. leopoliensis UTCC 100 *Synechococcus* sp. PCC 6803	Natural conjugation electroporation	Lindberg et al. (2010); Kaczmarzyk and Fulda (2010)

4.10 Conclusions

Although the use of chemical pesticides in agriculture is helpful in the increment of crop production, soil productivity, and products quality, it is also reflected in economic benefits, vector disease control, and in general, in public health. But approximately only 10 % of applied pesticides reach the target organism and rest of the applied pesticides is deposited into soil, water, and sediments which affects the nontarget organism in agro- and aquatic ecosystems besides affecting public health. For this reason, it is necessary to generate strategies for the removal of pesticide contamination from polluted sites, and the biological treatment is an important technology from an economical and environmental point of view for the cleanup of pesticide contamination.

The choice of the bioremediation strategy should be made on the basis of the type of pesticide, environment, and the target organisms present in the ecosystem. Since, the target organism is the only major concern and the information about features, advantages or disadvantages of target organisms can be helpful for better and successive bioremediation. Some parameters like pH, temperature, cell count, biomass growth rate, substrate bioavailability, and moisture, which are crucial for microbial population, can be addressed for bioremediation (Velázquez-Fernández et al. 2012). Moreover, it is important to understand the molecular mechanisms involved in enzymatic catalysis, which will be possible to design new alternatives and/or efficient tools for the treatment of pesticide residues or for the bioremediation of contaminated sites.

Use of cyanobacteria and microlage for the degradation of synthetic pesticides either by increasing the degradation capability of the cyanobacterial community to remove the pollutant is cost-effective and safe technology (Kumar and Singh 2016). Among the cyanobacterial genera, the high tolerance of some cyanobacterial genera toward synthetic pesticides resulted in colonized contaminated environments. It should also be kept in mind that cyanobacteria provide high product selectivity, simple catalyst preparation, and a recycling system.

Moreover, in implementing strategies to increase the efficiency of degradation, such as immobilization of cyanobacterial cells, we may have tools to decline the existence of obsolete pesticides and waste generated; it will reduce the danger of pesticides on the environment and health (Ortiz-Hernández et al. 2013). However, there is a suggestion that immobilization affects the cell's behavior, but many of the observations, particularly with respect to productivity are contradictory. It is therefore, there is a need to increase understanding on the effects of immobilization on cyanobacterial cell physiology and biochemistry. The leakage problem is one of the key concerns in cell immobilization since it obviates the primary purpose of delimiting viable cells in a confined matrix.

Despite the uncertainty regarding the development of GE algae as production strains, development of genetic tools is still imperative from a research standpoint. Understanding the basic biology that will inform such aspects as lateral gene transfer, potential for toxin production, potential for large-scale blooms and subsequent anoxic zone formation, and choice of cultivation methods in terms of organism containment, are very important.

References

Abdullah AR, Bajet CM, Matin MA, Nhan DD, Sulaiman AH (1997) Ecotoxicology of pesticides in the tropical paddy field system. Environ Toxicol Chem 16:59–70

Akhtar W, Sengupta D, Chowdhury A (2009) Impact of pesticide use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1):1–12

Alavanja MCR et al (2004) Health effects of chronic pesticide exposure – cancer and neurotoxicity. Annu Rev Public Health 25:155–197

Alexander M (1977) Introduction to soil microbiology, 2nd edn. Wiley Eastern Limited, New Delhi

Alexander M (1994) Biodegradation und bioremediation. Academic Press, San Diego, CA

Alexander M (1999) Biodegradation and bioremediation, 2nd edn. Academic Press, London

Araujo ASF, Monterio RTR, Abarkeli RB (2003) Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52:799–804

Armstrong RN (1994) Glutathione S-transferases: structure and mechanism of an archetypical detoxication enzyme. Adv Enzymol Relat Areas Mol Biol 69:1–44

Arunakumara KKIU, Walpola BC, Yoon MH (2013) Metabolism and degradation of glyphosate in aquatic cyanobacteria: a review. Afr J Microbiol Res 7(32):4084–4090

Asada Y, Miyake M, Miyake J, Kurane R, Tokiwa Y (1999) Photosynthetic accumulation of poly-(hydroxybutyrate) by cyanobacteria-the metabolism and potential for CO2 recycling. Int J Biol Macromol 25:37–42

Babu GS, Farooq M, Ray RS, Joshi PC, Viswanathan PN, Hans RK (2003) DDT and HCH residues in basmati rice (*Oryza sativa*) cultivated in Dehradun (India). Water Air Soil Pollut 144:149–157

Barcelo D, Hennion MC (1997) Trace determination of pesticides and their degradation products in water. Elsevier, Amsterdam, The Netherlands, p. 3

Barton JW, Kurtiz T, O'Connor LE, Ma CY, Maskarinee MP, Davison BH (2004) Reductive transformation of methyl parathion by cyanobacterium *Anabaena* sp., strain PCC 7120. Appl Microbiol Biotechnol 65:330–335

Bödeker W, Dümmler C (1993) Pestizide und Gesundheit, 2nd edn. Verlag C.F Müller, Karlsruhe

Burja AM, Banaigs B, Abou-Mansour E, Grant Burgess J, Wright PC (2001) Marine cyanobacteria-a prolific source of natural products. Tetrahedron 57:9347–9377

Burns RG (1975) Factors affecting pesticides loss from soil. In: Paul E A, AD ML (eds) Soil biochemistry, vol 4. Marcel Dekker, Inc., New York, pp 103–141

Caceres TP, Megharaj M, Naidu R (2008) Biodegradation of the pesticide fenamiphos by ten different species of green algae and cyanobacteria. Curr Microbiol 57(6):643–646

Capone DG, Burns JA, Montoya JP et al (2005) Nitrogen fixation by *Trichodesmium* spp.: an important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. Global Biogeochem Cycles 19(2):1–17

Chen W, Georgiou G (2002) Cell-surface display of heterologous proteins: from high throughput screening to environmental applications. Biotechnol Bioeng 5:496–503

Codd GA (1987) Immobilized micro-algae and cyanobacteria. Br Phycol Soc Newsl 24:1–5

Cohen Y (2001) Biofiltration-the treatment of fluids by microorganisms immobilized into the filter bedding material: a review. Bioresour Technol 77:257–274

Cork DJ, Krueger JP (1991) Microbial transformations of herbicides and pesticides. Adv Appl Microbiol 36:1–66

De-Bashan LE, Yoav Bashan Y (2010) Immobilized microalgae for removing pollutants: review of practical aspects. Bioresour Technol 101:1611–1627

Doggett SM, Rhodes RG (1991) Effects of a diazinon formulations on unialgal growth rates and phytoplankton diversity. Bull Environ Contam Toxicol 47:36–42

Dong G, Golden SS (2008) How a cyanobacterium tells time. Curr Opin Microbiol 11:541–546

Drum C (1980) Soil chemistry of pesticides. PPG Industries, Inc., Pittsburgh, PA

Dyhrman ST, Chapell PD, Haley ST, Moffett JW, Orchard ED, Waterbury JB (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature 439:68–71

El-Bestawy EA, Abd El-Salam AZ, Mansy AERH (2007) Potential use of environmental cyanobacterial species in bioremediation on lindane-contaminated effluents. Int Biodeter Biodegr 59:180–192

El-Nahhal Y, Awad Y, Safi J (2013) Bioremediation of acetochlor in soil and water systems by cyanobacterial Mat. Int J Geosci 4:880–890

EPA, 2012. What is a pesticide? http://www.epa.gov/opp00001/about/.

FAO (1989) International code of conduct on the distribution and use of pesticides, Rome.

Forget G (1993) Balancing the need for pesticides with the risk to human health. In: Forget G, Goodman T, de Villiers A (eds) Impact of pesticide use on health in developing countries. IDRC, Ottawa, p 2

Forlani G, Pavan M, Gramek M, Kafarski P, Lipok J (2008) Biochemical basis for a wide spread tolerance of cyanobacteria to the phosphonate herbicide glyphosate. Plant Cell Physiol 49:443–456

Freedman B (1995) Environmental ecology. Academic Press, New York

Gimsing AL, Borggaard OK, Jacobsen OS, Sørensen AJ (2004) Chemical and microbiological soil characteristics controlling glyphosate mineralization in Danish surface soils. Appl Soil Ecol 27:233–242

Glotfelty DE, Schomburg CJ (1989) Volatilization of pesticides from soil. In: Sawhney BL, Brown K, Madison WI (eds) Reactions and movements of organic chemicals in soil. Soil Science Society of America Special Publication, Madison, WI, p 181

González R, García-Balboa C, Rouco M, Lopez-Rodas V, Costas E (2012) Adaptation of microalgae to lindane: a new approach for bioremediation. Aquat Toxicol 109:25–32

Ha J, Engler CR, Lee SJ (2008) Determination of diffusion coefficients and diffusion characteristics for chlorferon and diethylthiophosphate in Ca-alginate gel beads. Biotechnol Bioeng 100(4):698–706

Ha J, Engler CR, Wild J (2009) Biodegradation of coumaphos, chlorferon, and diethylthiophosphate using bacteria immobilized in Ca-alginate gel beads. Bioresour Technol 100:1138–1142

Habib K, Kumar S, Manikar N, Zutshi S, Fatma T (2011) Biochemical effect of carbaryl on oxidative stress, antioxidant enzymes and osmolytes of cyanobacterium *Calothrix brevissima*. Bull Environ Contam Toxicol 87:615–620

Happe T, Schutz K, Bohme H (2000) Transcriptional and mutational analysis of the uptake hydrogenase of the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. J Bacteriol 182:1624–1631

Hatzios KK (1991) Biotransformation of herbicides in higher plants. In: Grover R, Cessna AJ (eds) Environmental chemistry of herbicides. CRC Press, Boca Raton, FL, pp 141–185

Hedges SB, Chen H, Kumar S, Wang DY, Thompson AS, Watanabe H (2001) A genomic timescale for the origin of eukaryotes. BMC Evol Biol 1(4):1–10

Hicks RJ, Stotzky G, Voris PV (1990) Review and evaluation of the effects of xenobiotic chemical on microorganisms in soil. Adv Appl Microbiol 35:195–253

Hirooka T, Nagase H, Hirata K, Miyamoto K (2006) Degradation of 2,4-dinitrophenol by a mixed culture of photoautotrophic microorganisms. Biochem Eng J 29:157–162

Ibrahim WM, Karam MA, El-Shahat RM, Adway AA (2014) Biodegradation and utilization of organophosphorus pesticide malathion by cyanobacteria. Biomed Res Int. Article ID 392682

Igbedioh SO (1991) Effects of agricultural pesticides on humans, animals and higher plants in developing countries. Arch Environ Health 46:218

Jha MN, Mishra SK (2005) Biological responses of cyanobacteria to insecticides and their insecticide degrading potential. Bull Environ Contam Toxicol 75(2):374–381

Kaczmarzyk D, Fulda M (2010) Fatty acid activation in cyanobacteria mediated by acyl-acyl carrier protein synthetase enables fatty acid recycling. Plant Physiol 152:1598–1610

Kanekar PP, Bhadbhade BJ, Deshpande NM, Sarnaik SS (2004) Biodegradation of organophosphorous pesticides. Proc Indian Natl Sci Acad B 70(1):57–70

Kennedy IR, Sanchez-Bayo F, Kimber SW, Hugo L, Ahmad N (2001) Off-site movement of endosulfan from irrigated cotton in New South Wales. J Environ Qual 30:683–696

Khasdan V, Ben-Dov E, Manasherob R, Boussiba S, Zaritsky A (2003) Mosquito larvicidal activity of transgenic *Anabaena* PCC 7120 expressing toxin genes from *Bacillus thuringiensis* subsp. *israelensis*. FEMS Microbiol Lett 227:189–195

Kodama T, Kuwatsuka S (1980) Factors for the persistence of parathion, methyl parathion and fenitrothion in seawater. J Pestic Sci 5:351–355

Kole RK, Bagchi MM (1995) Pesticide residues in the aquatic environment and their possible ecological hazards. J Inland Fish Soc India 27(2):79–89

Kulasooriya SA (2011) Cyanobacteria: pioneers of planet earth. Ceylon J Sci (Bio Sci) 40(2):71–88

Kumar A, Singh JS (2016) Microalgae and cyanobacteria biofuels: a sustainable alternate to crop-based fuels. In: Singh JS, Singh DP (eds) Microbes and environmental management. Studium Press Pvt. Ltd., New Delhi, pp 1–20

Kumar N, Bora A, Kumar R, Amb MK (2012) Differential effects of agricultural pesticides endosulfan and tebuconazole on photosynthetic pigments, metabolism and assimilating enzymes of three heterotrophic, filamentous cyanobacteria. J Biol Environ Sci 6(16):67–75

Kumar NJI, Amb MK, Kumar RN, Bora A, Khan SR (2013) Studies on biodegradation and molecular characterization of 2,4-D ethyl ester and pencycuron induced cyanobacteria by using GC-MS and 16S r DNA sequencing. Proc Int Acad Ecol Environ Sci 3(1):1–24

Kuritz T (1999) Cyanobacteria as agents for the control of pollution by pesticides and chlorinated organic compounds. J Appl Microbiol Symp Supplement 85:186S–192S

Kuritz T, Wolk CP (1995) Use of filamentous cyanobacteria for biodegradation of organic pollutants. Appl Environ Microbiol 61:234–238

Lal R, Pandey G, Sharma P, Kumari K, Malhotra S, Pandey R, Raina V, Kohler HPE, Holliger C, Jackson C, Oakeshott JG (2010) Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. Microbiol Mol Biol Rev 74:58–80

Lee SE, Kim JS, Kennedy IR, Park JW, Kwon GS, Koh SC, Kim JE (2003) Biotransformation of an organochlorine insecticide, endosulfan, by *Anabaena* Species. J Agric Food Chem 51:1336–1340

Lindberg P, Schütz K, Happe T, Lindblad P (2002) A hydrogen-producing, hydrogenase-free mutant strain of *Nostoc punctiforme* ATCC 29133. Int J Hydrogen Energy 27:1291–1296

Lindberg P, Park S, Melis A (2010) Engineering a platform for photosynthetic isoprene production in cyanobacteria, using *Synechocystis* as the model organism. Metab Eng 12:70–79

Lipok J, Owsiak T, Młynarz P, Forlani G, Kafarski P (2007) Phosphorus NMR as a tool to study mineralization of organophosphonates-The ability of *Spirulina* spp. to degrade glyphosate. Enzyme Microb Technol 41:286–291

Lipok J, Wieczorek D, Jewginski M, Kafarski P (2009) Prospects of in vivo 31P NMR method in glyphosate degradation studies in whole cell system. Enzyme Microb Technol 44:11–16

Lusta KA, Starostina NG, Fikhte BA (1990) Immobilization of microorganisms: cytophysiological aspects. In: De Bont JAM, Visser J, Mattiasson B, Tramper J (eds) Proceedings of an international symposium: physiology of immmobilized cells. Elsevier, Amsterdam, The Netherlands

Majewski MS, Capel PD (1995) Pesticides in the atmosphere: distribution, trends, and governing factors, Pesticides in the hydrologic system, vol Vol 1. Ann Arbor Press Inc, Ann Arbor, MI, p. 118

Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15:377–390

Manahan SE (2001) Fundamentals of environmental chemistry, 2nd edn. CRC Press LLC, Boca Raton

Marrs KA (1996) The functions and regulation of glutathione S-transferases in plants. Annu Rev Plant Physiol Plant Mol Biol 47:127–158

Martin H (1968) Pesticides manual. British Crop Protection Council, London

Martín M, Mengs G, Plaza E, Garbi C, Sánchez M, Gibello A, Gutiérrez F, Ferrer E (2000) Propachlor removal by *Pseudomonas* strain GCH1 in a immobilized-cell system. Appl Environ Microbiol 66(3):1190–1194

Masukawa H, Inoue K, Sakurai H (2007) Effects of disruption of homocitrate synthase genes on *Nostoc* sp. Strain PCC 7120 photobiological hydrogen production and nitrogenase. Appl Environ Microbiol 73:7562–7570

McNeely K, Xu Y, Bennette N, Bryant DA, Dismukes GC (2010) Redirecting reductant flux into hydrogen production via metabolic engineering of fermentative carbon metabolism in a cyanobacterium. Appl Environ Microbiol 76:5032–5038

Megharaj M, Venkateswarlu K, Rao AS (1987) Metabolism of monocrotophos and quinalphos by algae isolated from soil. Bull Environ Contam Toxicol 39:251–256

Megharaj M, Madhavi DR, Sreenivasulu C, Umamaheswari A, Venkateswarlu K (1994) Biodegradation of methyl parathion by soil isolates of microalgae and cyanobacteria. Bull Environ Contam Toxicol 53:292–297

Miyake M, Takase K, Narato M, Khatipov E, Schnackenberg J, Shirai M et al (2000) Polyhydroxybutyrate production from carbon dioxide by cyanobacteria. Appl Biochem Biotechnol 84:991–1002

Moreno-Garrido I (2008) Microalgae immobilization: current techniques and uses. Bioresour Technol 99:3949–3964

Moslemy P, Guiot SR, Neufeld RJ (2002) Production of size-controlled gellan gum microbeads encapsulating gasoline-degrading bacteria. Enzyme Microb Technol 30:10–18

Mostafa FIY, Helling CS (2001) Isoproturon degradation as affected by the growth of two algal species at different concentrations and pH values. J Environ Sci Health B 36(6):709–727

Murali O, Shaik G, Mehar SK (2014) Assessment of bioremediation of Cobalt and Chromium using cyanobacteria. Ind J Fund Appl Life Sci 4(1):252–255

Niederholtmeyer H, Wolfstadter BT, Savage DF, Silver PA, Way JC (2010) Engineering cyanobacteria to synthesize and export hydrophilic products. Appl Environ Microbiol 76:3462–3466

Nobles D, Brown R (2008) Transgenic expression of *Gluconacetobacter xylinus* strain ATCC 53582 cellulose synthase genes in the cyanobacterium *Synechococcus leopoliensis* strain UTCC 100. Cellul 15:691–701

O'Neil W, Raucher R, Wayzata MN (1998) Groundwater Policy Education Project, Groundwater public policy leaflet series #4: the costs of groundwater contamination.

Ortiz-Hernández ML, Sánchez-Salinas E, Olvera-Velona A, Folch-Mallol JL (2011) Pesticides in the environment: impacts and its biodegradation as a strategy for residues treatment. In: Stoytcheva M (ed) Pesticides - formulations, effects, fate. InTech, Rijeka, pp 551–574

Ortiz-Hernández ML, Sánchez-Salinas E, Dantán-González E, Castrejón-Godínez ML (2013) Pesticide biodegradation: mechanisms, genetics and strategies to enhance the process. In: Chamy R, Rosenkranz F (eds) Biodegradation - life of science. InTech, Rijeka

Orus MI, Marco E (1991) Disappearance of trichlorophon from cultures with different cyanobacteria. Bull Environ Contam Toxicol 47:392–397

Palanisami S, Prabaharan D, Uma L (2009) Fate of few pesticide metabolizing enzymes in marine cyanobacterium *Phormidium valderianum* BDU 20041 in perspective with chlorpyifos exposure. Pestic Biochem Physiol 94:68–72

Parkinson A (2001) Biotransformation of xenobiotics. In: Klaassen CD (ed) Casarett and Doull's toxicology: the basic science of poisons, 6th edn. McGraw-Hill, New York, NY, pp 133–224

Qiong LI, Qing T, Xudong XU, Hong GAO (2010) Expression of organophosphorous-degradation gene (opd) in aggregating and non-aggregating filamentous nitrogen-fixing cyanobacteria. Chinese J Oceanol Limnol 28:1248–1253

Racke KD, Skidmore MW, Hamilton DJ, Unsworth JB, Miyamoto J, Cohen SZ (1997) Pesticide fate in tropical soils. Pure Appl Chem 69:1349–1371

Ravi V, Balakumar H (1998) Biodegradation of the C-P bond in glyphosate by the cyanobacterium *Anabaena variabilis* L. J Sci Ind Res India 57:790–794

Ravindran CRM, Suguna S, Shanmugasundaram S (2000) Tolerance of oscillatoria isolates to agrochemicals and pyrethroid components. Indian J Exp Biol 38:402–404

Reddy BR, Sethunathan N (1985) Salinity and the persistence of parathion in flooded soil. Soil Biol Biochem 17:235–239

Reppas NB, Ridley CP (2010) Methods and compositions for the recombinant biosynthesis of n-alkanes. Patent US 7794969, Joule Unlimited, Inc., Washington, DC

Richins R, Mulchandani A, Chen W (2000) Expression, immobilization, and enzymatic characterization of cellulose-binding domain-organophosphorus hydrolase fusion enzymes. Biotechnol Bioeng 69:591–596

Roberts TR (1998) Metabolic pathways of agrochemicals-part 1: herbicides and plant growth regulators. The Royal Society of Chemistry, Cambridge, pp. 396–399

Roberts TR, Hutson DH (1999) Acephate: metabolic pathways of agrochemicals - part 2: insecticides and fungicides. The Royal Society of Chemistry, Cambridge, pp. 201–204

Robinson PK, Mak AL, Trevan MD (1986) Immobilized algae: a review. Process Biochem 21:122–126

Roessler PG, Chen Y, Liu B, Dodge CN (2009) Secretion of fatty acids by photosynthetic microorganisms in Patent US, Ed. Synthetic Genomics, United States

Savonen C (1997) Soil microorganisms object of new OSU service. Good Fruit Grower. http://www.goodfruit.com/archive/1995/6other.html

Singh PK (1973) Effect of pesticides on blue-green algae. Arch Microbiol 89:317–320

Singh DK (2008) Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Indian J Microbiol 48(1):35–40

Singh JS, Gupta VK (2016) Degraded land restoration in reinstating CH_4 sink. Front Microbiol 7(923):1–5

Singh JS, Abhilash PC, Singh HB, Singh RP, Singh DP (2011a) Genetically engineered bacteria: An emerging tool for environmental remediation and future research perspectives. Gene 480:1–9

Singh DP, Khattar JIS, Nadda J, Singh Y, Garg A, Kaur N, Gulati A (2011b) Chlorpyrifos degradation by the cyanobacterium *Synechocystis* sp. strain PUPCCC 64. Environ Sci Pollut Res 18:1351–1359

Singh JS, Singh DP, Dixit S (2011c) Cyanobacteria: an agent of heavy metal removal. In: Maheshwari DK, Dubey RC (eds) Bioremediation of pollutants. IK International Publisher Co., New Delhi, pp 223–243

Singh DP, Khattar JIS, Kaur M, Kaur G, Gupta M et al (2013) Anilofos tolerance and its mineralization by the Cyanobacterium *Synechocystis* sp. strain PUPCCC 64. PLoS One 8(1):e53445

Singh R, Singh P, Sharma R (2014) Microorganism as a tool of bioremediation technology for cleaning environment: a review. Proc Int Acad Ecol Environ Sci 4(1):1–6

Singh JS, Kumar A, Rai AN, Singh DP (2016) Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front Microbiol 7(529):1–19

Sode K, Yamamoto Y, Hatano N (1998) Construction of a marine cyanobacterial strain with increased heavy metal ion tolerance by introducing exogenic metallothioneins gene. J Mar Biotechnol 6:174–177

Subramanian G, Sekar S, Sampoornam S (1994) Biodegradation and utilization of organophosphorus pesticides by cyanobacteria. Int Biodegrad Biodetertor 33:129–143

Sun W, Chen Y, Liu L, Tang J, Chen J, Liu P (2010) Conidia immobilization of T-DNA inserted *Trichoderma atroviride* mutant AMT-28 with dichlorvos degradation ability and exploration of biodegradation mechanism. Bioresour Technol 101:9197–9203

Taha TH, Taha Alamri SA, Mahdy HM, Hafez EE (2013) The effects of various immobilization matrices on biosurfactant production using hydrocarbon (HC)-degrading marine bacteria via the entrapment technique. J Biol Sci 13:48–57

Takeshima Y, Takatsugu N, Sugiura M, Hagiwara H (1994) High-level expression of human superoxide dismutase in the cyanobacterium *Anacystis nidulans* 6301. Proc Natl Acad Sci U S A 91:9685–9689

Tao XQ, Lu GN, Liu JP, Li T, Yang LN (2009) Rapid degradation of phenanthrene by using Sphingomonas sp. GY2B immobilized in calcium alginate gel beads. Int J Environ Res Public Health 6:2470–2480

Terre Des Hommes (2011) Pestizide und Kinder. PAN Germany, Osnabrück

Thengodkar RRM, Sivakami S (2010) Degradation of Chlorpyrifos by an alkaline phosphatase from the cyanobacterium *Spirulina platensis*. Biodegradation 21:637–644

Topp E, Vallaeys T, Soulas G (1997) Pesticides microbial degradation and effect on microorganisms. In: Van Elsas JD, Trevors JT, Wellington EMH (eds) Modern soil microbiology. Mercel Dekker Inc., New York, pp 547–575

UNEP (2004) Childhood pesticide poisoning, Châtelaine

Urrutia I, Serra JL, Llama MJ (1995) Nitrate removal from water by *Scenedesmus obliquus* immobilized in polymeric foams. Enzyme Microb Technol 17:200–205

US EPA (2001) Source water protection practices bulletin: Managing small-scale application of pesticides to prevent contamination of drinking water. Office of Water, Washington, DC EPA 816-F-01-031

Velázquez-Fernández JB, Martínez-Rizo AB, Ramírez-Sandoval M, Domínguez-Ojeda D (2012) Biodegradation and bioremediation of organic pesticides. In: Soundarajan RP (ed) Pesticides – recent trends in pesticide residue assay. InTech, Rijeka

Vermaas WFJ (2001) Photosynthesis and respiration in cyanobacteria. Wiley, New York

Waliszewski SM, Carvajal O, Gomez-Arroyo S, Amador-Munoz O, Villalobos-Pietrini R, Hayward-Jones PM, Valencia-Quintana R (2008) DDT and HCH isomer levels in soils, carrot root and carrot leaf samples. Bull Environ Contam Toxicol 81:343–347

Walker WW (1976) Chemical and microbiological degradation of malathion and in an estuarine environment. J Environ Qual 5:210–216

Waskom R (1994) Best management practices for private well protection. Colorado State Univ. Cooperative Extension. http://hermes.ecn.purdue.edu:8001/cgi/convertwq?7488

Weber K (1976) Degradation of parathion in seawater. Water Res 10:237–241

Whitton BA, Potts M (2000) Introduction to the cyanobacteria. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic, Dordrecht, The Netherlands, pp 1–11

WWF (2002) Gefahr durch hormonell wirksame Pestizide und Biozide, Schadstoffe in Lebensmitteln, Garten und Haus. WWF-Fachbereich Meere und Küsten, Bremen

Yoshino F, Ikeda H, Masukawa H, Sakurai H (2007) High photobiological hydrogen production activity of a *Nostoc* sp. PCC 7422 uptake hydrogenase-deficient mutant with high nitrogenase activity. Marine Biotechnol 9:101–112

Yu R, Yamada A, Watanabe K, Yazawa K, Takeyama H, Matsunaga T et al (2000) Production of eicosapentaenoic acid by a recombinant marine cyanobacterium, *Synechococcus* sp. Lipids 35:1061–1064

Zacharia JT (2011) Identity, physical and chemical properties of pesticides. In: Stoytcheva M (ed) Pesticides in the modern world - trends in pesticides analysis. InTech, Rijeka

Chapter 5
Aromatic Plant–Microbe Associations: A Sustainable Approach for Remediation of Polluted Soils

Sanjeet K. Verma, Pragya Trivedi, Anand K. Gupta, and Rajesh Kumar Verma

Abstract Plant–microbe association is a key driving factor for proper functioning of an ecosystem. Microbes are being popularly used to facilitate plant growth and agricultural productivity as they are actively involved in decomposition of organic matter, biogeochemical cycling, and soil structure formation. In spite of these functions, current empirical studies support the use of microbes for bioremediation (bioaccumulation, bio-transformation, volatilization, etc.) of various pollutants in our environment. As food crops cannot be recommended for cultivation on polluted sites due to potential risks of pollutants' bioaccumulation, on the other hand, arable lands, cannot be utilized for cultivation of aromatic plants due to pressure of food demand. Hence, cultivation of aromatic crops on such contaminated sites will be a safe strategy as aromatic plants are stress-tolerant and their end product is essential oils, which are non-edible and remain free from pollutants. Reports suggest that the use of suitable plant–microbe association can be helpful in remediation of polluted sites as microbial secondary metabolites favor the plant growth, increase plant tolerance to pollutants, and also enhance the phytoextraction efficiency of plant by increasing the bioavailability of pollutants in rhizosphere. In this chapter, therefore, we review the available literature and discuss future perspectives on application of microbes in association with aromatic plants for remediation of heavy metal and xenobiotic polluted soils.

Keywords Aromatic Plants • Bioremediation • Microorganisms • Polluted soil • Sustainable agriculture

S.K. Verma (✉) • P. Trivedi • A.K. Gupta • R.K. Verma
Division of Agronomy and Soil Science, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, Uttar Pradesh, India
e-mail: sanjeet.kverma@gmail.com

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_5

5.1 Introduction

Soil pollution is a serious global problem and it is increasing at an alarming rate. Soil pollutants may be of different types like plastics, synthetic organic and inorganic chemicals, industrial effluents, municipal garbage, etc. Due to soil pollution, several problems are emerging such as health hazards to human and animals, loss of biodiversity, loss of soil fertility and productivity, and loss of natural resources due to contamination (Wall et al. 2015).

The physical, chemical, and biological methods are being implicated for remediation of polluted soil for long time back (Sims 1990). Due to economical and ecological constraints in physical and chemical methods, biological methods of remediation or bioremediation have been considered better strategy than the earlier two. The remediation of polluted sites through biological means is generally termed as bioremediation, either we use plants or microbes. In this chapter, therefore, for the convenience of the readers, biological remediation is divided into bioremediation (use of microbial system) and phytoremediation (use of plant system). Bioremediation is popularly known as the use of naturally occurring organisms, mainly microbes, to detoxify and degrade environmental contaminants. It is an effective approach, useful to clean up both heavy metals (Brierley 1990; Ghosh et al. 2015) and synthetic chemicals (Farhadian et al. 2008). Microbes produce organic chelates by decomposing the soil organic matter, which increase metal solubilization. They have capability to mobilize or immobilize the metals. Secondary metabolites and siderophore produced by the microbes take part in ion exchange mechanism through which they solubilize and bind the metals (Gadd 2004; Rajkumar et al. 2010).

The term 'Phytoremediation' comprised of two words 'phyto' (*Gk.*) means plant and 'remedium' (*Latin*) means to remove an evil (Cunningham et al. 1996). Phytoremediation is a cost-effective, in situ, low-cost, ecologically benign, socially accepted technology to remediate polluted soils (Radzali et al. 2015; Garbisu et al. 2002; Weber et al. 2001). Phytoremediation may take one of the several forms like phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization (He and Yang 2007). This technology can be applied to both organic and inorganic pollutants present in soil (Salt et al. 1998). Plants can be used to extract, sequester, and/or detoxify a wide variety of environmental contaminants. Phytoremediation could be more appropriate technique at a large scale to restore land and water that have been polluted by human activities.

There is plethora of literature supporting the use of edible crops for phytoremediation. But the cultivation of edible crops leads to biomagnification of xenobiotics in humans and other animals via food chain. Alternatively, some researchers have proposed use of aromatic plants for remediation purposes (Gupta et al. 2013; Verma et al. 2014; Pandey and Singh 2015). Aromatic plants are a class of plants which produce or exude aroma due to the presence of volatile aromatic compounds in its essential oil. The commercial product of aromatic plants, i.e. essential oil, is extracted through hydro-distillation or steam distillation process (Clevenger 1928). Essential oil is of great industrial importance for high-grade perfumes, culinary,

toiletries, cosmetics, insect repellants, and food processing industries. Since these plants are unpalatable and tolerant to different stress conditions, they become the most suitable candidates for phytoremediation of polluted soils (Gupta et al. 2013; Verma et al. 2014).

Healthy plant–microbe interaction is fundamental for any ecosystem. Plant–microbe associations may be positive as well as negative. Microbes remain associated to every part of plants (Rosenblueth and Martinez-Romero 2006). These microbes consist of specific beneficial associations as well as detrimental pathogenic ones (Raaijmakers et al. 2009). Beneficial microbes are applied as biofertilizers, biopesticides or plant protection products, rhizoremediators, phytostimulators, or stress controllers, for example, plant growth promoting bacteria like *Bacillus* and *Pseudomonas*, the symbiotic Arbuscular Mycorrhizal Fungi (AMF), and fungus *Trichoderma* (Pereg and McMillan 2015; Dobbelaere et al. 2003; Alabouvette et al. 2006; Nehra and Choudhary 2015). Pathogens of plant species include viruses, bacteria, fungi, or nematodes.

In this chapter, we have emphasized on the applicability of aromatic plant–microbe association for remediation of polluted soils. Although bioremediation using plants and microbes alone is a good approach in this concern, it has some constraints and needs more improvisations. As the plants are not able to grow well under certain stress conditions until the belowground microflora provides growth support (nutrients, hormones, siderophores, etc.), microbes can perform better in association with plants. Use of appropriate plant–microbe associations would be a better alternative as both of them complement each other for proper functioning and growth. Aromatic plants are the best suited for cultivation on polluted land; at the same time, use of resistant microbes can enhance the remediation of polluted sites. The utilization of aromatic plants–microbe associations could be a novel technique for remediation of polluted sites.

5.2 Soil Pollution

Soil pollution or contamination is the presence of any undesirable inorganic or organic chemicals or other alterations in the natural composition of soil, which change the normal functioning of soil ecosystem. Like all other cases of pollutions, soil pollution is also largely due to human activities. Some main contributors of soil pollution are: (a) agrochemicals, (b) industrial effluents, (c) urban, and (d) nuclear wastes (Rieuwerts 2015; Gavrilescu et al. 2015).

- *Agrochemicals*: Maintaining soil quality is the major need for sustainable agriculture. Nowadays, intensive farming is adapted to meet the increasing demand of food (Matson et al. 1997). In these practices, pesticides, fertilizers, and fumigants are used indiscriminately, which adversely affect soil environment (Edwards et al. 1998). These pollutants are either toxic or they convert into toxic entities after transformation. Higher amount of NPK fertilizers also lead to low

quality of crops grown over the years. Pesticides such as aldrin, malathion, dieldrin, chlorinated hydrocarbons, organophosphates, furadan, etc. are less prone to biodegradation and alter the physical, chemical, and biological properties of soil (Edwards and Thompson 1973). Some residues of these chemicals retain in the soil and later taken by the plants and enter into the food chain.

- *Industrial effluents*: Industries involved in manufacture/production of textiles, dyes, soaps, detergents, drugs, cement, rubber, metal, paper, etc. release considerable amount of solid, semi-solid, and liquid wastes in the environment (Islam et al. 2006). These chemicals pollute the land, nearby manufacturing plants as well as the place where the waste has been taken to dispose-off (Kuperman 1996). Industrial wastes mainly comprise pollutants like heavy metals, inorganic chemicals, volatile organic compounds (VOCs), polychlorinated biphenyls (PCBs), hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), etc. These pollutants have an adverse effect on soil's physical, chemical, and biological properties and eventually reduce the crop production (Islam et al. 2006).
- *Urban wastes*: The amount of municipal or urban waste has increased tremendously in recent decades and new disposal techniques are required to overcome this problem (Madrid et al. 2007). It includes large amount of biodegradable materials such as papers, food residues, animal wastes, wooden pieces, carcasses, plant twigs, sweepings, etc. as well as non-biodegradable materials such as plastic items, glass pieces, stone/cement pieces, etc. (Bogomolov et al. 1996).

5.3 Remediation of Polluted Soils

Soil remediation deals with the removal of pollutants or contaminants from polluted sites. The soil has innate capability to attenuate any undesirable and harmful changes via different physico-chemical and biological processes (Suthersan 1999; Gianfreda and Rao 2004). Most of these natural processes are ineffective, except abiotic oxidation, hydrolysis, and biodegradation, since they have the capability to destroy the pollutants and transform them into harmless products (Gianfreda and Rao 2004). To remediate the pollutants, several physical and chemical methods were also developed by the researchers, but they are forfeited towards economical and/or ecological front. Therefore, biological strategies using plants (phytoremediation) and microbes (bioremediation) seem to be a better approach to remediate the soil in a sustainable manner (Chibuike and Obiora 2014) (Fig. 5.1).

5.3.1 Bioremediation

Bioremediation refers to the use of naturally occurring organisms mainly microbes to detoxify and degrade environmental contaminants (Vidali 2001). It has received increasing attention as an effective approach to clean up polluted environments. Microorganisms are useful in both heavy metals (Banerjee et al. 2015;

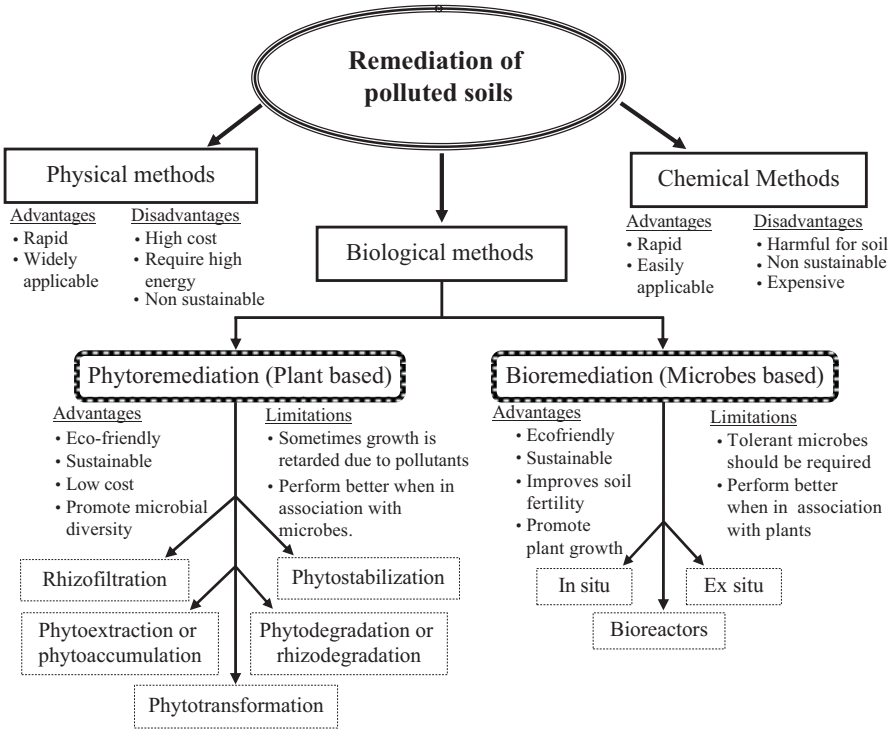


Fig. 5.1 Schematic diagram showing different strategies for remediation of polluted soils

Dixit et al. 2015) and organic compounds' polluted soils (Chen et al. 2015; Farhadian et al. 2008). They decompose the soil organic matter and produce organic ligands which lead to increased metal solubilization and altered speciation of metal or metalloids. They have capability to mobilize or immobilize the metals. Secondary metabolites and siderophore produced by a number of microbes are also able to bind toxic cationic metals or desorb anionic species via ligand exchange (Gadd 2004; Rajkumar et al. 2010). Apart from this, many soil bacteria have the capability to degrade toxic organic compounds like PAHs including fluorene, phenanthrene, anthracene, fluoranthene pyrene, etc. (Sayara et al. 2015), DDT (Foght et al. 2001), and compounds from oil spills (Atlas 1991). There are a number of microbes that are able to degrade various xenobiotic compounds such as refrigerants herbicides, pesticides, solvents, and other organic compounds. Wang et al. (2015) reported a bacterial strain WJ4 (genus Rhodococcus) that had a strong ability to degrade Di-2-ethylhexyl phthalate (DEHP-high-molecular-weight phthalate ester (PAE) that has been widely used in the manufacture of polyvinylchloride) in both liquid culture and soil.

Yateem et al. (1998) reported that white rot fungi (*Phanerochaete chrysosporium*, *Pleurotus ostreatus*, and *Coriolus versicolor,* etc.) possess the ability to degrade a wide spectrum of environmental pollutants like petroleum hydrocarbon using peroxidase enzymes. In another report, *Mycobacteria*, *Sphingomonas,* and white rot fungi are considered as capable of degrading polycyclic aromatic hydrocarbons

(PAHs) (four or more fused ring) degrading organisms due to the presence of lignin peroxidases and P450 monooxygenase (Harayama 1997). Use of effective microbial consortia is also a promising method for remediating polluted soils. Kumar et al. (2008) reported that consortia of *Ochrobacterum* sp., *Arthrobacter* sp., and *Burkholderia* sp. are able to degrade xenobiotics like α-endosulfan and β-endosulfan. Mixture of heavy metal-resistant bacteria comprised of different species of *Enterobacter*, *Enterobacter*, *Stenotrophomonas*, *Providencia*, *Chryseobacterium*, *Comamonas*, *Ochrobactrum,* and *Delftia* isolated from the activated sludge were found more efficient in removing heavy metals (Bestawy et al. 2013).

Microbes have a range of potentials to remediate polluted sites; however, modifications by use of genetic and metabolic engineering can further increase the effectiveness and efficiency of them (Samanta et al. 2002). Sayler and Ripp (2000) showed potential of genetically engineered microorganisms (GEMs) to remediate soil, water and activated sludge, etc. With the help of modern molecular techniques like fluorescence in situ hybridization (FISH), in situ PCR, and quantitative PCR, etc., we can detect and identify those bacteria, pathways, enzymes, etc., which are solely related to the degradation of inorganic and organic contaminants (Singh et al. 2008; Timmis and Pieper 1999; Pieper and Reineke 2000; Chen et al. 1999). To monitor changes in microbial community composition during remediation process, certain nucleic acid-based molecular techniques like denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) are found to be very useful (Iwamoto and Nasu 2001).

Apart from this, Christofi and Ivshina (2002)suggested that use of biosurfactant-producing bacteria or surface-active compounds (biosurfactant) can increase the efficiency of organic and metal-contaminated site remediation. However, Harayama (1997) found the microbes having capability to degrade PAHs and suggested that it is important to explain the effect of these biosurfactants on biodegradation through mathematical models. Malik (2004) focuses on the applicability of growing bacterial/fungal/algal cells for metal removal that are isolated from contaminated sites and possess excellent capability of metal scavenging. Therefore, bioremediation is a widely accepted environment- and economy-friendly method for remediation (Iwamoto and Nasu 2001).

5.3.2 Phytoremediation

The term ‘Phytoremediation’ comprised of two words ‘phyto’ (Gk.) means plant and ‘remedium’ (Latin) means to remove an evil (Cunningham et al. 1996). Phytoremediation may take one of the several forms like phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization. Phytoextraction refers to the processes in which plants are used to concentrate metals from the soil into the roots and shoots of the plant. Rhizofiltration is the use of plant roots to absorb, concentrate, or precipitate metals from effluents. Phytostabilization is the use of plants to reduce the mobility of heavy metals through absorption and precipitation by plants,

thus reducing their bioavailability. Phytovolatilization is the uptake and release into the atmosphere of volatile materials such as mercury- or arsenic-containing compounds (He and Yang 2007). Phytoremediation is cost-effective, in situ, non-intrusive, low-cost, aesthetically pleasing, ecologically benign, socially accepted technology to remediate polluted soils (Radzali et al. 2015; Garbisu et al. 2002; Weber et al. 2001). To remove pollutants, plants use several biophysical and biochemical processes, like adsorption, translocation, accumulation, transformation, and mineralization (Gupta et al. 2013).

This technology can be applied to both organic and inorganic pollutants present in soil (Salt et al. 1998). Plants can be used to extract, sequester, and/or detoxify a wide variety of environmental contaminants. This field is generating great excitement because phytoremediation techniques may offer the only effective means of restoring the hundreds of thousands of square miles of land and water that have been polluted by human activities.

5.4 Suitability of Aromatic Crops for Phytoremediation

A number of investigations and opinions support the suitability of aromatic plants for phytoremediation potential (Singer et al. 2003; Gupta et al. 2013; Verma et al. 2014). Aromatic plants are such a gift of nature which can be used as a cash crop under polluted sites because their end product, i.e. essential oil, is extracted through hydro-distillation process. In hydro-distillation process, the essential oil form an azeotropic mixture with water, from which the oil is further separated. In this process, the essential oil components volatilize when come in contact with steam; the non-volatile components (like pollutants and heavy metals) do not volatilize and hence the essential oil remains free from any contamination (Gupta et al. 2013). During crop production, certain precautions should be kept in mind to avoid physical contact of pollutants. The essential oil is of great industrial importance for high-grade perfumes, culinary, toiletries, cosmetics, insect repellants, and food processing industries.

Unlike edible crops, aromatic plants are free from the risk of being eaten by the animals or humans due to their bad taste and fragrance; hence, the contaminants will not enter into food chain. There are several views proposing that the cultivation of aromatic plants will be a safe strategy to phytoremediate the polluted sites along with economical benefit and without any ecological, economical, and health hazard (Gupta et al. 2013; Verma et al. 2014). Aromatic plants are better stress-tolerant and perennial in nature, hence such crops have high value with low input.

Rosmarinus officinalis flourished well in bio-wastes containing heavy metals such as Pb, Cr, Cd, and Ni without compromising the biomass production and essential oil yield (Cala et al. 2005). *R. officinalis* can be grown in the Pb, As, Sb, Zn, and Cu contaminated soil because the bioaccumulation factors of the aerial parts were less than 1 (Affholder et al. 2013). While at higher concentration of metals, the growth of the plant decreases (Gaida et al. 2013). *Mentha piperita* and *Ocimum basilicum* yields were not affected by the Cu, Pb, and Cd at the concentration of 60

and 150 ppm; however, the yield and growth of *Anethum graveolens* was affected by Cu. Oils of these three plants do not contaminate with heavy metals, but oil composition was slightly changed (Zheljazkov et al. 2006). Licina et al. (2014) demonstrated different genotypes of basil (*Ocimum* sp.) for their mineral nutrients and heavy metal accumulation potential. Genotypes have shown different accumulation behavior for different elements. *Marrubium vulgare*, *Melissa officinalis,* and *Origanum heracleoticum* were tested against Cd, Pb, Cu, Mn, and Zn metals. The heavy metals accumulation was different in different plant parts; the maximum accumulation of Cd, Pb, and Cu was in roots, while of Mn and Zn was found in leaves without availability of pollutant in essential oil, a commercial product (Zheljazkov et al. 2008). *Origanum majorana* inoculated with arbuscularmycorrhizal fungi (AMF) performs well on Cd and Pb polluted soils (Hristozkova et al. 2015). The heavy metal-tolerant AMF strains reduce the accumulation of heavy metals in the plant. The content of major constituents changes with the inoculation of AMF. Antioxidant activity in the plant also increases with AMF due to the increase of phenolic content (Fig. 5.3).

Vetiver (*Crysopogon zizanioides*), an aromatic grass having essential oil in its roots, was found suitable for phytostabilization and phytoextraction of heavy metal like As, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn. Vetiver also has some chelating agents which help in binding these toxic elements and promote biodegradation of organic pollutants like 2,4,6-trinitroluene, phenol, ethidium bromide, benzo[a]pyrene, atrazine, etc. Vetiver differentially accumulates heavy metals in root and shoots (Danh et al. 2009).

Cultivation of aromatic plants in association with organic manures or natural chemicals, which have high cation exchange capacity, was also tested under polluted soils. Danh et al. (2011) have also demonstrated the effect of Ca on growth, essential oil yield, and composition of vetiver (*C. zizanioides*) on Pb (4000 ppm) contaminated soils. Danh et al. (2011) concluded that calcium addition decreases the accumulation of Pb in the root and shoot of vetiver, but up to a certain limit (2000 ppm). The accumulation of Pb was >22 % more in roots than shoots of vetiver. *Ocimum gratissimum* performed differently towards the Cd and Zn metal uptake due to the addition of cow manure and hydroxyaptite. They found that, with the addition of cow manure, the uptake of Cd was decreased, while Zn uptake was decreased due to hydroxyapetite (Chaiyarat et al. 2011). Vermicompost-assisted cultivation of chammomile (*Matricaria chamomilla*) accumulates more heavy metals (Ni, Cd) as compared to in absence of vermicompost. The accumulation of heavy metals in flowers of the crop does not affect the quality and quantity of main component of commercial importance (Chand et al. 2012) (Table 5.1).

Soil properties like soil microbial biomass nitrogen (SMBN) and soil enzymes change due to the addition of heavy metals-rich tannery sludge in soil, which was used for growing *Tagetes minuta*. At moderate level of heavy metal concentration, SMBN and urease activity found higher, while at higher sludge concentration these parameters decrease. The heavy metals were found higher in roots as compared to shoot and there be no contamination in essential oil (Patel and Patra 2014). In aromatic plants,

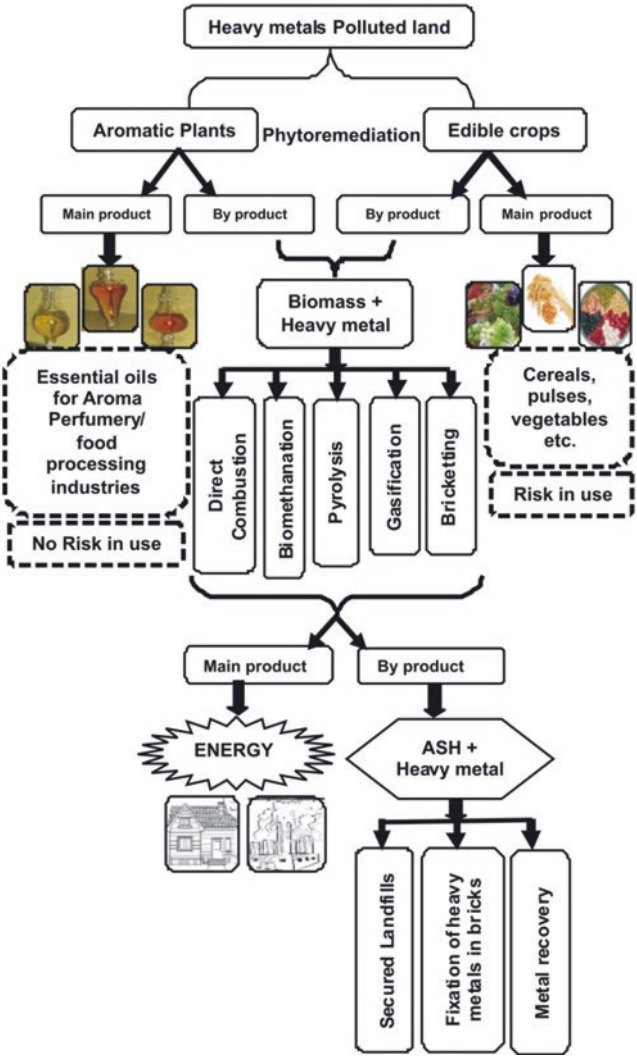


Fig. 5.2 Schematic diagram showing suitability of aromatic crops for phytoremediation as compared to edible crops [adapted with permission from Gupta et al. (2013) Copyright (2013) American Chemical Society]

the concentration and activity of antioxidants starts due to stress created by the pollutants. Glutathione activity increases in the aromatic plants (*T. minuta*, *M. spicata*, *O. basilicum,* and *Pelargonium graveolens*) with increasing heavy metal dose given in the form of tannery sludge (Patel and Patra 2015). *M. spicata* have shown higher glutathione and glutathione reductase activity as compared to other tested species. Chand et al. (2015) in another experiment found that Cd and Pb uptake by *O. basilicum* was higher at 20 t ha^{-1}use of sludge. However, Cr accumulation was increasing with dose

Table 5.1 The remediation potential of different aromatic plants against various pollutants

S.No	Aromatic plants	Grown against pollutants	Activity/physiological changes	Reference
1.	*Rosmarinus officinalis*	Pb, Cr, Cd and Ni; As, Sb, Zn and Cu	Tolerant, bioaccumulation factor is less than 1. At higher level, plant growth decreases	Cala et al. (2005); Affholder et al. (2013); Gaida et al. (2013)
2.	*Anethum graveolens* *Mentha piperita* *Ocimum basilicum*	Cd, Pb, and Cu	*Mentha* and *Ocimum* were more tolerant; *Ocimum* genotypes have different accumulation behavior	Zheljazkov et al. (2006); Licina et al. (2014)
3.	*Marrubium vulgare* *Melissa officinalis* *Origanum heracleoticum*	Cd, Pb, Cu, Mn, and Zn	Cd, Pb, and Cu were maximum absorbed in roots, while Mn and Zn were maximum absorbed in leaves.	Zheljazkov et al. (2008)
4.	*Chrysopogon zizanioides*	As, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn like 2,4,6-trinitroluene, phenol, ethidium bromide, benzo[a] pyrene, atrazine	Releases chelating agents which bind the metals. Degrades organic pollutants	Danh et al. (2009, 2010)
5.	*Tagetes minuta*, *Mentha spicata*, *Ocimum basilicum* *Pelargonium graveolens*	Tannery sludge (Cr, Pb)	At moderate level, SMBN and enzyme activity increases. The antioxidants activity increased in plants	Patel and Patra (2014, 2015); Chand et al. (2015)
6.	*Cymbopogon martinii*	Cr, Ni, Pb, Cd	Uptake order Cr>Ni>Pb>Cd	Pandey et al. (2015)
7.	*Mentha piperita*	Coal fly ash (Cr, As, Cd, Pb, Hg, Se, PAH)	at moderate level growth and yield increases	Kumar and Patra (2012)
8.	*Mentha piperita*	Cd, Pb	Plant can tolerate medium range of used metals.	Amirmoradi et al. (2012)
9.	*Lavandula vera*	Cd, Pb, Zn	A hyperaccumulator of Pb and accumulator of Cd and Zn	Angelova et al. (2015)
10.	*Chrysopogon zizanioides*	Pb	Ca decreases Pb accumulation, the accumulation in roots was more than shoots.	Danh et al. (2011)

S.No	Aromatic plants	Grown against pollutants	Activity/physiological changes	Reference
11.	*Origanum majorana*	Cd, Pb	AMF reduce the accumulation of metals in the plant.	Hristozkova et al. (2015)
12.	*Ocimum gratissimum*	Cd, Zn	Uptake of metals decreased due to cow manure and hydroxyapetite	Chaiyarat et al. (2011)
13.	*Matricaria chamomilla*	Ni, Cd	Vermcompost enables hyperaccumulation of metals.	Chand et al. (2012)
14.	*Cymbopogon flexuosus*	Municipal waste water (Cr, Ni, Pb, and Cd)	Growth increased in waste water-treated plots	Lal et al. (2013)
15.	*Mentha crispa*	Pb	Oil yield increases, carvone concentration in essential oil increases	Sa et al. (2015)
16.	*Mentha spicata* *Ocimum basilicum* *Ocimum citriodurum*	Cu, Cd, and Pb	Chemical composition of oil changes, accumulation of Cd decreases the biomass; silicate reduces the accumulation of Cd.	Kunwar et al. (2015); Putwattana (2008)
17.	*Ocimum basilicum* *Origanum vulgare*	Cd, Pb	Antioxidants defense system activates	Stancheva et al. (2014)
18.	*Ocimum tenuiflorum, Ocimum gratissimum*	Cd, Zn;As	*O. gratissimum* had a higher tolerance; at low level of As oil yield increases	Suebsimma (2008); Siddiqui et al. (2013)
19.	*Rorippa globosa* *Rorippa palustris*	Cd	Species-specific behavior	Wei et al. (2012)

up to 50 t ha^{-1}. The yield of essential oil decreases at higher dose of sludge application. The essential oil composition of *O. basilicum* changes due to Cu, Cd, and Pb contamination, while that of *M. spicata* does not (Kunwar et al. 2015). Cd-contaminated hydroponic culture of *O. basilicum* and *O. citriodurum* results indicate that biomass was higher in *O. basilicum,* while Cd accumulation was higher in *O. citriodurum*. Another experiment in which cow dung manure was used suggests that biomass productivity and Cd accumulation have increased manyfold. The use of silicate increases the biomass production, while Cd accumulation was decreased (Putwattana 2008). *O. basilicum* (sweet basil) and *Origanum vulgare* (Oregano) both accumulate Cd, while Pb was accumulated only in oregano shoots. Due to heavy metals, the leaf blades thickness of both plant increased. In basil stomatal conductance, gaseous exchange, and transpiration increased, while water-use efficiency decreases. While in oregano gas exchange and transpiration were reduced, but stomatal conductance and water-use efficiency increases due to heavy metals. Antioxidant defense system (glutathione peroxidase, quaiacol peroxidase, glutathione S-transferase, and glutathione reductase) triggered in both the plants due to heavy metals treatments (Stancheva et al. 2014). *Ocimum tenuiflorum* (holy basil) and *O. gratissimum* (African basil) were tested for HM tolerance towards Cd, Zn. *O. gratissimum* had a higher tolerance for Cd and Zn (Suebsimma 2008). *O. gratissimum* accumulates more Arsenic (As) than *O. basilicum* and *O. tenuiflorum*. Growth of all the species was reduced due to availability of As, while at lower As concentration the essential oil yield was increased and the constituents were not affected (Siddiqui et al. 2013).

Rorippa spp. (yellow cresses) shows species-specific behavior towards Cd metal. *R. globosa* was hyperaccumulator, while *R. palustris* was not. Root lengths, total root surface areas, and total root volumes of *R. globosa* were not affected by Cd, while all the growth parameters of *R. palustris* were reduced (Wei et al. 2012). The performance of two varieties of *Cymbopogon martinii* has been tested by Pandey et al. (2015) and found that Trishna variety was better performer than PRC-1 in heavy metal-contaminated soil, for improving soil properties and plant growth. In *C. martinii,* the heavy metal uptake was in order of Cr>Ni>Pb>Cd, in both root and shoot. Translocation factor was <1, while bioconcentration factor was >1 in case of all tested heavy metals. There was no contamination found in essential oil.

The coal fly ash is hazardous due to the presence of toxic heavy metals like Cr, As, Cd, Pb, Hg, Se, polycyclic aromatic hydrocarbons, and several other pollutants (Verma et al. 2014). Fly ash at 50 % concentration gives positive results over growth and yield of *M. piperita*. Fly ash along with oil cake of Jatropha supported growth and development of plant and essential oil yield (Kumar and Patra 2012). The ratios of heavy metal containing municipal waste water and ground water were used for irrigation of lemon grass (*Cymbopogon flexuosus*). The yield of lemon grass was 16 % more in waste water-treated plots than normal water-irrigated plots, and the contamination in essential oil was also below the permissible limits of present heavy metals (Cr, Ni, Pb, and Cd) (Lal et al. 2013).

With increasing concentration of cadmium (Cd; 10–100 ppm) and lead (Pb; 100–1500 ppm), there was a decrease in biomass production, plant height, leaf area, and number of leaves per plant and essential oil content of peppermint can tolerate

medium range of Cd and Pb (Amirmoradi et al. 2012). Lavender (*Lavandula spica*) acts as a hyperaccumulator of Pb and accumulator of Cd and Zn and can be cultivated successfully in heavy metal-polluted area without compromising the yield and quality of essential oil (Angelova et al. 2015). Pb contamination increases the oil production in *Mentha crispa*. It tolerates higher concentration of Pb, rather it is not a Pb hyperaccumulator sp.; yet it accumulates in root and aerial parts. The chemical composition of essential oil also changes due to Pb treatment and the major component, carvone, increased from 39.3 % (control) to 90 % (Sa et al. 2015).

5.5 Plant–Microbe Associations with Reference to Aromatic Plants

Plants are highly colonized with diverse group of microorganisms, which may reside in the rhizosphere, phyllosphere, or as endophytes (Rosenblueth and Martinez-Romero 2006). These microbes consist of different beneficial associations as well as detrimental pathogenic ones (Raaijmakers et al. 2009). Beneficial microbes are applied as biofertilizers, biopesticides or plant protection products, rhizoremediators, phytostimulators, as reductants or stress controllers (Rajendran et al. 2008; Pereg and McMillan 2015; Dobbelaere et al. 2003; Alabouvette et al. 2006; Nehra and Choudhary 2015). Secondary metabolites of aromatic plants have diverse effects on soil microbial community. The application of PGPRs and mycorrhiza in the aromatic crop field may enhance growth of plants through positive effect of secondary metabolites on soil microbes, and in return, this may increase essential oil production (Banchio et al. 2008). The microbes which are pathogenic to normal crops may behave differently with aromatic plants because there are many broad spectrum pathogens which do not cause disease to aromatic plants (Bennett and Wallsgrove 1994). The cotton seeds inoculated with aromatic compounds (citral and benzaldehyde) along with PGPR increase plant growth and reduce disease incidence (Bauske et al. 1997) (Fig. 5.3).

Aromatic plants are rich in secondary metabolites, which belong to diverse chemical groups. Use of plant residues (pine needles, eucalyptus leaves) containing secondary metabolites was found to enhance the microbes which degrade polychlorinated biphenyls (PCB) (Hernandez et al. 1997). Later on, it was found that plant secondary metabolites like terpenoids (carvone, carvone, cumene, carvacrol, thymol, limonene, cymene, trans-cinnamic acid, etc.) and flavonoids (myrcetin) support growth of different microbes (*Ralstonia eutropha, Rhodococcus opacus, Burkh-olderia cepacia,Corynebacterium* sp.) that degrade/transform various xenobiotics (PCB, PAH, toluene, phenol, naphthalene, trichloroethane, etc.) (Singer et al. 2003; Gilbert and Crowley 1997). The a-hydroxylase enzyme could effectively be induced by secondary metabolites for enhancing biotransformation of a number of compounds and can be potentially used for bioremediation (Singer et al. 2003). Jones et al. (2001) manipulated the *cyp101* gene to increase substrate range of substrate, mainly pollutants.

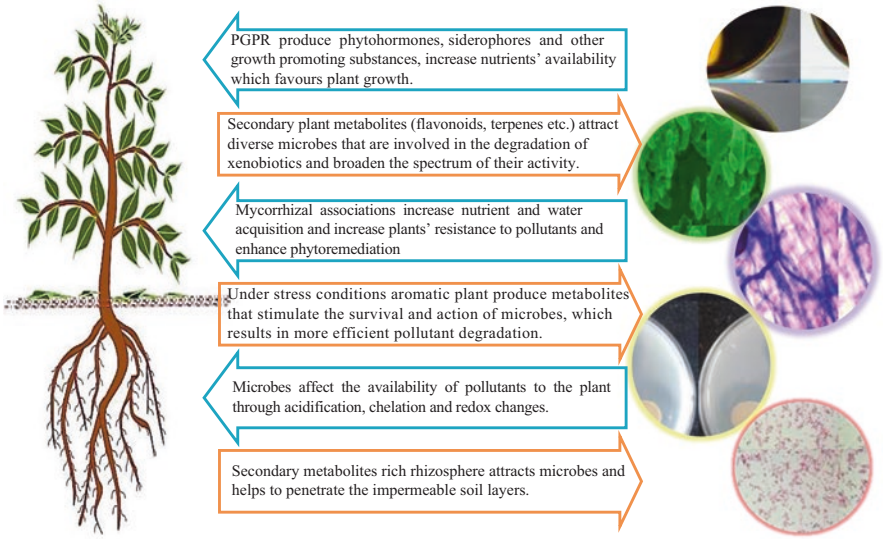


Fig. 5.3 Schematic representation of aromatic plant–microbe association under polluted environment

The cultivation of aromatic plants on polluted sites will be ecologically safe, economically viable as well as sustainable technology than other known methods. The agricultural land is a constraint to meet the rising food demand and increase in polluted land is creating a menace to this problem. The essential oil production in aromatic plants also increases with stress condition, which will benefit the growers, while in stress the productivity of edible crops decreases. The polluted land can be converted into normal agricultural land through long-term cultivation of aromatic crops (Gupta et al. 2013) along with beneficial microbes (Banchio et al. 2008). Hence, we propose cultivation of aromatic plants in place of edible crops or other plant crop.

5.6 Conclusion

Aromatic plant–microbe association can be exploited for remediation of polluted sites as well as achieving several ecological, economical, and societal/cultural benefits. Aromatic plants can add up secondary plant metabolites into the polluted soil, which enhances the activity of xenobiotics-degrading microbes. The degradation or transformation of pollutants and phytoextraction by plant will reduce the stress in the soil ecosystem, which will further enhance the growth of flora and fauna. The use of aromatic plants in place of edible crops as well as any other non-commercially important crop gives an extra advantage. As edible crops grown under polluted environment are ecologically not safe, while the other plants may be hyper-accumulator

but, without economical importance, is of no use. Phytoremediation technology needs commercialization by harnessing the phyto-microbial diversity for more attention. There is an urgent need to develop technological interventions at policy level, which will promote the cultivation of aromatic plants on polluted and degraded land sites. Moreover, the use of aromatic plants has additional benefits (ecosystem services) for farmers as well as for society than conventional technologies used for phytoremediation.

Acknowledgments Sanjeet K. Verma (CSIR-Senior Research Fellow) and PragyaTrivedi (UGC-Junior Research Fellow) express sincere thanks to the Council of Scientific and Industrial Research, New Delhi, India, and University Grants Commission, New Delhi, India, respectively, for financial assistance in the form of Fellowships. We are also thankful to KripalSingh and UmeshPankaj for their assistance. Financial support from CSIR (CSIR Network Project: INDEPTH, BSC-0111), New Delhi, is gratefully acknowledged.

References

Affholder MC, Prudent P, Masotti V, Coulomb B, Rabier J, Nguyen-The B, Laffont-Schwob I (2013) Transfer of metals and metalloids from soil to shoots in wild rosemary (*Rosmarinus officinalis* L.) growing on a former lead smelter site: human exposure risk. Sci Total Environ 454:219–229

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant pathogens: the European situation. Eur J Plant Pathol 114:329–341

Amirmoradi S, Moghaddam PR, Koocheki A, Danesh S, Fotovat A (2012) Effect of cadmium and lead on quantitative and essential oil traits of peppermint (*Mentha piperita* L.). Not Sci Biol 4(4):101–109

Angelova VR, Grekov DF, Kisyov VK, Ivanov KI (2015) Potential of Lavender (*Lavandula vera* L.) for phytoremediation of soils contaminated with heavy metals WASET. Int J Biol Biomol Agric Food Biotechnol Eng 9(5):479–486

Atlas RM (1991) Microbial hydrocarbon degradation–bioremediation of oil spills. J Chem Technol Biotechnol 52(2):149–156

Banchio E, Bogino PC, Zygadlo J, Giordano W (2008) Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana* L. Biochem Syst Ecol 36(10):766–771

Banerjee G, Pandey S, Ray AK, Kumar R (2015) Bioremediation of heavy metals by a novel bacterial strain *Enterobacter cloacae* and its antioxidant enzyme activity, flocculant production, and protein expression in presence of lead, cadmium, and nickel. Water Air Soil Pollut 226(4):1–9

Bauske EM, Backman PA, Harper KM, Brannen PM, Rodríguez-Kábana R, Kloepper JW (1997) Effect of botanical aromatic compounds and seed-surface pH on growth and colonization of cotton plant growth-promoting rhizobacteria. Biocontrol Sci Technol 7(3):415–422

Bennett RN, Wallsgrove RM (1994) Tansley Review No. 72. Secondary metabolites in plant defence mechanisms. New Phytol 127:617–633

Bestawy EE, Helmy S, Hussien H, Fahmy M, Amer R (2013) Bioremediation of heavy metal-contaminated effluent using optimized activated sludge bacteria. Appl Water Sci 3(1):181–192

Bogomolov DM, Chen SK, Parmelee RW, Subler S, Edwards CA (1996) An ecosystem approach to soil toxicity testing: a study of copper contamination in laboratory microcosms. Appl Soil Ecol 4:95–105

Brierley CL (1990) Bioremediation of metal-contaminated surface and groundwaters. Geomicrobiol J 8(3–4):201–223

Cala V, Cases MA, Walter I (2005) Biomass production and heavy metal content of *Rosmarinus officinalis* grown on organic waste-amended soil. J Arid Environ 62(3):401–412
Chaiyarat R, Suebsima R, Putwattana N, Kruatrachue M, Pokethitiyook P (2011) Effects of soil amendments on growth and metal uptake by *Ocimum gratissimum* grown in Cd/Zn-contaminated soil. Water Air Soil Pollut 214(1–4):383–392
Chand S, Pandey A, Patra DD (2012) Influence of vermicompost on dry matter yield and uptake of Ni and Cd by chamomile (*Matricaria chamomilla*) in Ni-and Cd-polluted soil. Water Air Soil Pollut 223(5):2257–2262
Chand S, Singh S, Singh VK, Patra DD (2015) Utilization of heavy metal-rich tannery sludge for sweet basil (*Ocimum basilicum* L.) cultivation. Environ Sci Pollut R 22(10):7470–7475
Chen W, Brühlmann F, Richins RD, Mulchandani A (1999) Engineering of improved microbes and enzymes for bioremediation. Curr Opin Biotechnol 10(2):137–141
Chen M, Xu P, Zeng G, Yang C, Huang D, Zhang J (2015) Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs. Biotechnol Adv 33(6 pt 1):745–755
Chibuike GU, Obiora SC (2014) Bioremediation of hydrocarbon-polluted soils for improved crop performance. Int J Environ Sci 4:5
Christofi N, Ivshina IB (2002) Microbial surfactants and their use in field studies of soil remediation. J Appl Microbiol 93:915–929
Clevenger JT (1928) Apparatus for the determination of volatile oil. J American Asso 7:3–46
Cunningham SD, Huang JW, Chen J, Berti WR (1996) Phytoremediation of contaminated soils: Progress and promise. In: Abstracts of papers of the American Chemical Society, vol 212. American Chemical Society, Washington, DC, p 87-agro
Danh LT, Truong P, Mammucari R, Tran T, Foster N (2009) Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. Int J Phytoremediation 11(8):664–691
Danh LT, Truong P, Mammucari R, Foster N (2010) Economic incentive for applying vetiver grass to remediate lead, copper and zinc contaminated soils. Int J Phytoremediation 13(1):47–60
Danh LT, Truong P, Mammucari R, Foster N (2011) Effect of calcium on growth performance and essential oil of vetiver grass (*Chrysopogon zizanioides*) grown on lead contaminated soils. Int J Phytoremediation 13(1):154–165
Dixit R, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7(2):2189–2212
Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149
Edwards CA, Thompson AR (1973) Pesticides and the soil fauna. In: Residue reviews. Springer, New York, pp 1–79
EdwardsCA,KnackerT, PokarzhevskiiA (1998) The prediction of the fate and effects of pesticides in the environment using tiered laboratory soil microcosms. Proceedings of the Brighton Pesticide Conference, Pests and Diseases AC-1, pp267–272
Farhadian M, Vachelard C, Duchez D (2008) Christian in situ bioremediation of monoaromatic pollutants in groundwater: a review. Bioresour Technol 99:5296–5308
Foght J, April T, Biggar K, Aislabie J (2001) Bioremediation of DDT-contaminated soils: a review. Bioremed J 5:225–246
Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119
Gaida M, Landoulsi NR, Rejeb MN, Smiti S (2013) Growth and photosynthesis responses of *Rosmarinus officinalis* L. to heavy metals at Bougrine mine. Afr J Biotechnol 12(2):150–161
Garbisu C, Hernandez-Allica J, Barrutia O, Alkorta I, Becerril JM (2002) Phytoremediation: a technology using green plants to remove contaminants from polluted areas. Rev Environ Health 17:75–90

Gavrilescu M, Demnerová K, Aamand J, Agathos S, Fava F (2015) Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. N Biotechnol 32(1):147–156

Ghosh A, Ghosh Dastidar M, Sreekrishnan TR (2015) Recent advances in bioremediation of heavy metals and metal complex dyes: review. J Environ Eng C4015003

Gianfreda L, Rao MA (2004) Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzyme Microb Technol 35:339–354

Gilbert ES, Crowley DE (1997) Plant compounds that induce polychlorinated biphenyl biodegradation by Arthrobacter sp. strain B1B. Appl Environ Microbiol 63:1933–1938

Gupta AK, Verma SK, Khan K, Verma RK (2013) Phytoremediation using aromatic plants: a sustainable approach for remediation of heavy metals polluted sites. Environ Sci Technol 47(18):10115–10116

Harayama S (1997) Polycyclic aromatic hydrocarbon bioremediation design. Curr Opin Biotechnol 8(3):268–273

He ZL, Yang XE (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci B 8(3):192–207

Hernandez BS et al (1997) Terpene-utilizing isolates and their relevance to enhanced biotransformation of polychlorinated biphenyls in soil. Biodegradation 8:153–158

Hristozkova M, Geneva M, Stancheva I, Boychinova M, Djonova E (2015) Aspects of mycorrhizal colonization in adaptation of sweet marjoram (*Origanum majorana* L.) grown on industrially polluted soil. Turkish J Biol 39:461–468

Islam MO, Khan HR, Das AK, Akhtar MS, Oki Y, Adachi T (2006) Impacts of industrial effluents on plant growth and soil properties. Soil Environ 25(2):113–118

Iwamoto T, Nasu M (2001) Current bioremediation practice and perspective. J Biosci Bioeng 92:1–8

Jones JP, O'Hare EJ, Wong LL (2001) Oxidation of polychlorinated benzenes by genetically engineered CYP101 (cytochrome P450cam). Eur J Biochem 268(5):1460–1467

Kumar KV, Patra DD (2012) Alteration in yield and chemical composition of essential oil of *Mentha piperita* L. plant: effect of fly ash amendments and organic wastes. Ecol Eng 47:237–241

Kumar M, Lakshmi CV, Khanna S (2008) Biodegradation and bioremediation of endosulfan contaminated soil. Bioresour Technol 99:3116–3122

Kunwar G, Pande C, Tewari G, Singh C, Kharkwal GC (2015) Effect of heavy metals on terpenoid composition of *Ocimum basilicum* L. and *Mentha spicata* L. J Essent Oil Bear Pl 18(4):818–825

Kuperman RG (1996) A hierarchical approach to ecological assessment of contaminated soils at Aberdeen Proving Ground, USA. In: Van Straalen NM, Krivolutsky DA (eds) Bioindicator systems for soil pollution, NATO ASI Series, vol 16. Kluwer Academic Publishers, Dordrecht, pp 197–212

Lal K, Yadav RK, Kaur R, Bundela DS, Khan MI, Chaudhary M, Meena RL, Dar SR, Singh G (2013) Productivity, essential oil yield, and heavy metal accumulation in lemon grass (*Cymbopogon flexuosus*) under varied wastewater–groundwater irrigation regimes. Ind Crop Prod 45:270–278

Licina VĐ, Jelačić SĆ, Beatović DV, Antić-Mladenović SB (2014) Mineral composition of different basil (*Ocimum* spp.) genotypes. Hem Ind 68(4):501–510

Madrid F, López R, Cabrera F (2007) Metal accumulation in soil after application of municipal solid waste compost under intensive farming conditions. Agr Ecosyst Environ 119:249–256

Malik A (2004) Metal bioremediation through growing cells. Environ Int 30(2):261–278

Matson PA, Parton WJ, Power AG, Swift MJ (1997) Agricultural intensification and ecosystem properties. Science 277(5325):504–509

Nehra V, Choudhary M (2015) A review on plant growth promoting rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. J Appl Nat Sci 7(1):540–556

Pandey VC, Singh N (2015) Aromatic plants versus arsenic hazards in soils. J Geochem Explor 157:77–80

Pandey J, Chand S, Pandey S, Patra DD (2015) Palmarosa [*Cymbopogon martinii* (Roxb.) Wats.] as a putative crop for phytoremediation, in tannery sludge polluted soil. Ecotoxicol Environ Saf 122:296–302

Patel A, Patra DD (2014) Influence of heavy metal rich tannery sludge on soil enzymes vis-à-vis growth of *Tagetes minuta*, an essential oil bearing crop. Chemosphere 112:323–332

Patel A, Patra DD (2015) Effect of tannery sludge amended soil on glutathione activity of four aromatic crops: *Tagetes minuta*, *Pelargonium graveolens*, *Ocimum basilicum* and *Mentha spicata*. Ecol Eng 81:348–352

Pereg L, McMillan M (2015) Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems. Soil Biol Biochem 80:349–358

Pieper DH, Reineke W (2000) Engineering bacteria for bioremediation. Curr Opin Biotechnol 11:262–270

Putwattana N (2008) Effects of cow manure and silicate fertilizer on uptake and accumulation of cadmium in *Ocimum basilicum*. Mahidol University, Doctoral dissertation

Raaijmakers JM, Paulitz CT, Steinberg C, Alabouvette C, Moenne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321(1–2):341–361

Radzali NRSM, Kadir WRWA, Shariff SM, Nawahwi MZ, Wakid SA, Jaafar Z, Rahim MI (2015) Phytoremediation: environmental-friendly clean up method. World 5(2):16–22

Rajendran G, Sing F, Desai AJ, Archana G (2008) Enhanced growth and nodulation of pigeon pea by co-inoculation of *Bacillus* strains with *Rhizobium* spp. Bioresour Technol 99:4544–4550

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149

Rieuwerts J (2015) The elements of environmental pollution. Routledge

Rosenblueth M, Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19:827–837

Sa RA, Sá RA, Alberton O, Gazim ZC, Laverde A Jr, Caetano J, Amorin AC, Dragunski DC (2015) Phytoaccumulation and effect of lead on yield and chemical composition of Mentha crispa essential oil. Desalin Water Treat 53(11):3007–3017

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Biol 49(1):643–668

Samanta SK, Singh OV, Jain RK (2002) Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends Biotechnol 20(6):243–248

Sayara T, Čvančarová M, Cajthaml T, Sarrà M, Sánchez A (2015) Anaerobic bioremediation of pah–contaminated soil: assessment of the degradation of contaminants and biogas production under thermophilic and mesophilic conditions. Environ Eng Manag J 14(1):153–165

Sayler S, Ripp S (2000) Field applications of genetically engineered microorganisms for bioremediation processes Gary. Curr Opin Biotechnol 11:286–289

Siddiqui F, Krishna SK, Tandon PK, Srivastava S (2013) Arsenic accumulation in *Ocimum* spp. and its effect on growth and oil constituents. Acta Physiol Plant 35(4):1071–1079

Sims RC (1990) Soil remediation techniques at uncontrolled hazardous waste sites. J Air Waste Manage Assoc 40(5):704–732

Singer AC, Crowley DE, Thompson IP (2003) Secondary plant metabolites in phytoremediation and biotransformation. Trends Biotechnol 21(3):123–130

Singh S, Kang SH, Mulchandani A, Chen W (2008) Bioremediation: environmental clean-up through pathway engineering. Curr Opin Biotechnol 19:437–444

Stancheva I, Geneva M, Markovska Y, Tzvetkova N, Mitova I, Todorova M, Petrov P (2014) A comparative study on plant morphology, gas exchange parameters, and antioxidant response of *Ocimum basilicum* L. and *Origanum vulgare* L. grown on industrially polluted soil. Turkish J Biol 38(1):89–102

Suebsimma R (2008) Influence of soil amendments on growth and cadmium accumulation in *Ocimum gratissimum* (African Basil) and *O. tenuiflorum* (Holy Basil). Doctoral dissertation, Mahidol University

Suthersan SS (1999) In situ bioremediation. Remediation engineering: design concepts. CRC Press, Boca Raton

Timmis KN, Pieper DH (1999) Bacteria designed for bioremediation. Trends Biotechnol 17(5):201–204

Verma SK, Singh K, Gupta AK, Pandey VC, Trivedi P, Verma RK, Patra DD (2014) Aromatic grasses for phytomanagement of coal fly ash hazards. Ecol Eng 73:425–428

Vidali M (2001) Bioremediation an overview. Pure Appl Chem 73(7):1163–1172

Wall DH, Nielsen UN, Six J (2015) Soil biodiversity and human health. Nature 528:69–76

Wang J, MY Z, Chen T, Zhu Y, Teng Y, Y-M L, Christie P (2015) Isolation and identification of a di-(2-ethylhexyl) phthalate-degrading bacterium and its role in the bioremediation of a contaminated soil. Pedosphere 25(2):202–211

Weber O, Scholz R, Bühlmann R, Grasmück D (2001) Risk perception of heavy metal 89 soil contamination and attitudes toward decontamination strategies. Risk Anal 21(5):967–977

Wei S, Li Y, Zhan J, Wang S, Zhu J (2012) Tolerant mechanisms of Rorippa globosa (Turcz.) Thell. hyperaccumulating Cd explored from root morphology. Bioresour Technol 118:455–459

Yateem A, Balba MT, Al-Awadhi N, El-Nawawy AS (1998) White rot fungi and their role in remediating oil-contaminated soil. Environ Int 24(1):181–187

Zheljazkov VD, Craker LE, Xing B (2006) Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. Environ Exp Bot 58(1):9–16

Zheljazkov VD, Jeliazkova EA, Kovacheva N, Dzhurmanski A (2008) Metal uptake by medicinal plant species grown in soils contaminated by a smelter. Environ Exp Bot 64(3):207–216

Chapter 6
Cyanobacteria-Mediated Heavy Metal Remediation

Vidya Dhar Pandey

Abstract Heavy metals constitute toxic, non-biodegradable and persistent environment pollutants which adversely affect all life forms, including humans, and cause ecological damage. The detrimental effects of heavy metals on living organisms are attributable to their action on a number of cellular and biochemical processes, biomolecules and structures in living organisms, including human beings. In humans, they are known to cause various patho-physiological disorders of hepatic, renal, respiratory and gastrointestinal system. The biotoxicity of heavy metals depends on their concentration, bioavailability, chemical forms and duration of exposure. Globally, the ever-increasing contamination of aquatic bodies and soil by heavy metals (e.g. Cd, Hg, Ag, As, Pb, Ni, Cr, Cu, Zn) owing to various anthropogenic activities is an issue of serious concern and challenge. Bioremediation of heavy metals, employing various microorganisms, including cyanobacteria (blue–green algae), has been recognized as a cheaper, more effective and an eco-friendly alternative to the conventional physico-chemical remediation methods. Because of their tremendous adaptability and effective protective mechanisms against various abiotic stresses, cyanobacteria colonize and inhabit diverse terrestrial and aquatic habitats, including extreme and polluted ones. Various cyanobacterial species possess efficient heavy metal removal capabilities from aqueous solutions. They produce metal-binding proteins (metallothioneins) and metal-sequestering agents (e.g. exopolysaccharides). The bioremoval of heavy metals by cyanobacteria is mediated by biosorption and bioaccumulation. Cyanobacteria, because of their ubiquity, abundance, rapid growth rate, simple growth requirements, heavy metal tolerance and removal, and amenability to controlled laboratory culture and immobilization are the promising candidates for the bioremediation of heavy metal pollutants.

Keywords Heavy metals • Cyanobacteria • Bioremediation • Biosorption • Bioaccumulation

V.D. Pandey (✉)
Department of Botany, Government Post-Graduate College,
Rishikesh, Dehradun, Uttarakhand 249201, India
e-mail: vdpandey2@yahoo.co.in

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_6

6.1 Introduction

Excessive contamination of aquatic and terrestrial environments by heavy metals, such as cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), chromium (Cr), arsenic (As), silver (Ag), copper (Cu) and zinc (Zn) discharged as a result of industrial and other anthropogenic activities is an issue of major concern. Heavy metals are toxic, non-biodegradable, persistent and biomagnifiable environmental pollutants which adversely affect growth, development and survival of all life forms—microbes, plants, animals and humans. They are known to be major risk factor for human health. Although the term 'heavy metal' has been redefined over the years, it mostly refers to the metallic elements with density greater than 5 g/cm^3 and high atomic weight (Duffus 2002). They are mainly discharged from various mining and industrial sites and find way into the food chain and drinking water. Among 90 naturally occurring elements found in earth's crusts, nearly 53 are heavy metals (Weast 1984). Bioremediation of heavy metal-bearing industrial effluents and wastewater using the potential of various microorganisms, including cyanobacteria (blue–green algae), to sequester and remove various toxic heavy metals efficiently is viewed as a cheaper, more effective, eco-friendly and technologically simple alternative to the conventional physico-chemical remediation methods which are known to have various limitations.

Cyanobacteria (blue–green algae) are an ancient, morphologically diverse and widely distributed group of photosynthetic prokaryotes which resemble Gram-negative bacteria in cellular structure and green plants in oxygenic (O_2-evolving) photosynthesis (Stanier and Cohen-Bazire 1977; Castenholz and Waterbury 1989; Schopf 2000). They are classified by bacteriologists as the class Oxyphotobacteria in the eubacterial division Gracilicutes and by phycologists as the class Cyanophyceae or Myxophyceae in the algal division Cyanophyta (Carr and Whitton 1982; Castenholz and Waterbury 1989; Murray 1989). Historically, cyanobacteria were classified as algae (blue–green algae) based on the international code of botanical nomenclature (Oren 2004). After the establishment of their prokaryotic nature, their nomenclature was proposed to be governed by the international code of nomenclature of bacteria (Stanier et al. 1978). Comprising 150 genera with more than 2000 species, they exhibit remarkable diversity in their morphology, which may be unicellular, colonial or filamentous (branched or unbranched) (Van den Hoek et al. 1995). Because of their unique physiology and metabolism, they are considered to be highly productive and efficient biological system. Both ecologically and economically, they are important organisms with varied implications and applications (Waterbury et al. 1979; Patterson 1996; Pandey et al. 2007; Abed et al. 2009; Gupta et al. 2013). Due to their potential applications in diverse fields, viz. agriculture, aquaculture, human nutrition, bioenergy and biofuels, pharmaceuticals and pollution control, they have received considerable attention world wide. Owing to their tremendous adaptability to varying environmental conditions as well as effective protective mechanisms against various abiotic stresses (e.g. desiccation, salinity, ultraviolet radiation, heavy metals, high light intensity, oxidative and extremes of

temperature), they colonize, grow and survive in various types of terrestrial and aquatic (freshwater and marine) habitats, including those with extreme conditions and contaminated with various pollutants (Tandeau de Marsac and Houmard 1993; Turner and Robinson 1995; Potts 1999; Ehling-Schulz and Scherer 1999). Cyanobacteria are important primary producers in aquatic ecosystems, many of which are contaminated with various heavy metals. Many cyanobacterial species are able to tolerate elevated concentrations of various heavy metals due to the presence of well-developed and effective metal resistance mechanisms, and possess efficient heavy metal removal or sequestration capabilities from aqueous solution (Wilde and Benemann 1993; Fiore and Trevors 1994; Fiore et al. 1998; Robinson et al. 2000). Heavy metal tolerance and bioremoval capabilities as well as various intrinsic qualities and merits of cyanobacteria make them promising agents of bioremediation for the mitigation of heavy metal pollutants.

6.2 Heavy Metals as Pollutants

Heavy metals have gained notoriety as hazardous environmental pollutants with varying degree of toxicities to living organisms. With pronounced level biotoxicity and ecotoxicity, they adversely affect different levels of biological organization from biomolecules to ecosystem. Various industrial, agricultural, domestic, medical and technological applications as well as production and processing of heavy metals or heavy metal-containing compounds have resulted in their extensive distribution and accumulation in the environment, contributing significantly to the pollution of water, soil and air. Because of their prevalence and toxicity, they are the agents of public health significance. Unlike organic pollutants, they cannot be degraded and, therefore, persist in the environment indefinitely. Hg, Cd and their compounds have been placed under ‘black list’, the list of most dangerously toxic compounds, and other heavy metals, viz. Zn, Cu, Ni, Cr, Pb, Co, etc. and their compounds, which are regarded as less dangerous pollutants, have been included in the ‘grey list’ by European Economic Community (EEC) (McEldowney et al. 1993). Because of their toxicities, these heavy metals, along with other pollutants, have been included in EPA (Environmental Protection Agency) list of ‘priority pollutants’ (McEldowney et al. 1993). Although some heavy metals, such as Cu, Zn, Co, Ni and Se, are essential to living organisms in trace amounts as they are important constituents of several key enzymes and metalloproteins, and play roles in maintenance of metabolism, they are extremely toxic at higher concentrations. Heavy metals like Hg, Cd, As, Ag and Pb are not known to perform any biological function in metabolism of living organisms and are considered as non-essential metals (Duruibe et al. 2007). Although heavy metals are released from natural (weathering and volcanoes) and anthropogenic sources, their discharge and environmental accumulation due to various anthropogenic sources or activities (e.g. smelting, mining, tanning, use of pesticides, automobiles) is many times greater than those from natural sources (Nriagu and Pacyna 1988). In aquatic and terrestrial environment, heavy metals exist as

hydrated ionic species or they form complexes with inorganic and organic ligands or associated with colloids and suspended particulate matter. The behaviour and toxicity of heavy metals are closely related to their position in periodic table. The toxicity of a heavy metal towards a living organism depends upon its concentration, bioavailability, chemical forms or chemical speciation and duration of exposure. Moreover, the pH of water and soil is known to control their mobility, bioavailability and toxicity. For instance, acidification of aquatic bodies by acid rain exacerbates the problem of heavy metal toxicity (McEldowney et al. 1993). Heavy metals present in aquatic and terrestrial environment may enter into food chain and tend to bioaccumulate in living organisms (Grimanis et al. 1978; Rayms-Keller et al. 1998).

The detrimental or biotoxic effects of heavy metals are due to their action on a number of cellular and biochemical processes, biomolecules and structures in living organisms. Being systemic toxicants, they may cause a wide variety of pathophysiological conditions or induce multiple organ damage in animals and humans, such as liver damage, renal dysfunction, pulmonary edema, bronchitis, osteomalacia, osteoporosis, arthritis and neurological damage. Moreover, few of them, viz. Cd, Cr and As, are known to be carcinogenic (Fan and Harding-Barlow 1987; Bencko 1987; Hayes 1997; Zweig et al. 1999; Costa and Klein 2006). Human exposure to heavy metals occurs through contamination of food, air and water. Occupational exposure to various heavy metals is known to occur in many occupational or industrial settings (Sorahan and Waterhouse 1985; Pauls et al. 2003). Excessive accumulation of heavy metals in the environment may cause ecological damage with adverse effects on ecosystem functions and biodiversity. Their plausible ecological impacts include reduction in species diversity and abundance, disruption of nutrient cycling, inhibition of microbial or decomposer activities, slowing down of decomposition of dead biomass, and emergence and dominance of metal-tolerant ecotypes and species.

6.3 Microbial Bioremediation

The increasing level of contamination of environment by toxic heavy metals necessitated the development of cost-effective and eco-friendly remediation technologies for the mitigation or removal of heavy metal pollutants. The conventional technologies based on physico-chemical methods or processes, such as precipitation, ion-exchange, membrane separation, chemical oxidation/reduction and reverse-osmosis, are known to be expensive, technologically complex and non-eco-friendly, and loose effectiveness when heavy metals are present in very low concentrations (Volesky 1994; Kapoor and Viraraghavan 1995). Bioremediation of heavy metal-polluted soil and water, employing plants (phytoremediation) and microorganisms (microbial bioremediation or microbial remediation) is gaining importance as a cost-effective, technologically simple, efficient and eco-friendly alternative to the conventional physico-chemical remediation methods. Bioremediation is the process of removal, degradation or detoxification of toxic environmental pollutants using

biological activities or capacities of living organisms, especially plants and microbes, which may be indigenous to a contaminated site or introduced from elsewhere. The contaminants or pollutants, depending on their chemical nature and properties as well as on the metabolic capacities or activities of organisms involved, are either completely or partially removed from the ambient environment or degraded into products which may be harmless or less harmful. Due to ubiquitous distribution in environment, tremendous adaptability to environmental conditions, fast growth rate and metabolic versatility of microorganisms, microbial bioremediation has attracted more attention as compared to phytoremediation. Microbes from taxonomically different groups, viz. bacteria, cyanobacteria, microalgae and fungi (yeasts and molds), are important bioremediation agents. The importance of bioremediation as a sustainable method for mitigation and control of various environmental pollutants, including heavy metals, and restoration of contaminated sites or ecosystems is widely accepted. Bioremediation can be applied directly at the site of contamination (in situ) or away from it (ex situ). In the latter case, the contaminated material (i.e. soil or water) is removed from the original site to another place for treatment. In order to accelerate the process of bioremediation, promising microorganisms, natural or genetically engineered, are added or inoculated to the contaminated site. This is referred to as bio-augmentation. If naturally occurring or indigenous microbial population is induced to proliferate by the addition of inorganic or organic microbial nutrients and/or by the adjusting certain physico-chemical factors, such as temperature, pH, moisture and oxygen, optimally, this is called bio-stimulation.

Microbial cells can sequester and accumulate metals essential for their growth and metabolism as well as those with unknown physiological or metabolic roles. Microbial remediation of heavy metals is widely advocated due to certain merits, such as rapid kinetics of metal removal and removal of metal ions from very dilute aqueous solutions (De Philippis et al. 2003). Both prokaryotic (e.g. bacteria and cyanobacteria) and eukaryotic (e.g. microalgae and fungi) microbes have been reported as effective agents for heavy metal bioremediation (Brady and Duncan 1994; Kapoor and Viraraghavan 1995; Blanco et al. 1999; Vieira and Volesky 2000; De Philippis et al. 2003; Gaur and Adholeya 2004; Rao et al. 2005; Wang and Chen 2006). The large surface area to volume ratio of microbes confers them selective advantage to interact efficiently with metals and inorganic nutrients in the environment. The underlying mechanisms of microbial remediation of heavy metals include biosorption, bioaccumulation and chemical transformation. The term biosorption can be defined as the metabolically passive physico-chemical process which occurs naturally in certain microbial cells or biomass, leading to the binding, concentration or immobilization of heavy metal ions on to the cell surface, primarily cell wall. Being a passive process, it does not involve the expenditure of metabolic energy and occurs in both dead (inactive) and living (active) cells or biomass. Due to sequestration of metals from the aqueous solution of even very low or dilute concentrations, the process is considered to be efficient. The processes which are generally implicated in biosorption include chelation, complexation, co-ordination and ion-exchange. Cell wall, the first cellular structure of microbes that comes in contact with the metal ions present in soil and water, contains a wide variety of polymeric

constituents with negatively charged chemical groups, such as carboxyl, carbonyl, hydroxyl, sulfhydryl, sulfonate and phosphate, which have significant potential for metal binding due to their high affinity for metal cations (Kuyucak and Volesky 1988; Vieira and Volesky 2000). In many microbes, extracellular polymeric substances, e.g. exopolysaccharides (EPS), which lies outside the cell wall, contributes significantly to metal biosorption. Microbial biosorbents, both viable (living) and non-viable (non-living) biomass of various microbes, can be employed for the decontamination of heavy metal-containing industrial effluents and wastewater (Volesky 1994; Gupta et al. 2000). The metal sorption capacity of microbial biosorbents varies with the type and species of the organism as well as with the nature and properties of metal ions. Additionally, factors like temperature and pH strongly influence the biosorption of metals. The variation in metal-binding properties of different microorganisms can be attributed to the difference in their cell wall structure and composition. In addition to the use of microbial biosorbents for the removal of heavy metals from industrial effluents and wastewater, they can be used for the recovery of valuable and commercially important metals or elements, such as gold, silver, platinum and uranium after appropriate treatment (e.g. pH adjustment and addition of ligands) (Nakajima et al. 1982; Darnall et al. 1986; Brierley et al. 1986; Kuyucak and Volesky 1988; Chakarborty et al. 2009). Bioaccumulation refers to the intracellular uptake or accumulation of heavy metals from the ambient environment. As opposed to biosorption, it is an energy-dependent active process that occurs only in the living microbial cells. It can be defined as the process by which the intracellular concentration of a chemical in an organism achieves a level that exceeds its concentration in the surrounding environment. Due to high degree of metal resistance, many microbes can accumulate and tolerate elevated concentrations of heavy metals (Nies 1999). Microbial cells are known to possess metal uptake systems which facilitate the intracellular transport of various heavy metal ions (Nies 1999). In chemical transformation, a toxic heavy metal is converted into a non-toxic or less toxic form by the metabolic or enzymatic activities of microorganisms. Microbial transformation of metals mostly involves reactions like oxidation, reduction, methylation and demethylation (Chirwa and Wang 1997; Lloyd 2003; Barkay et al. 2003).

Few microbial biosorbents are known to be commercialized for the removal and recovery of heavy metals. These include AlgaSORB™, AMT-BIOCLAIM™ and BIO-FIX which are prepared by immobilization of specific biomass (Michalak et al. 2013). AlgaSORB™, a potent algal biosorbent, is composed of non-living biomass of *Chlorella vulgaris* (unicellular green alga) immobilized in silica gel polymer that was developed by Bio-recovery Systems, Inc. (Las Cruces, NM, USA) (Darnall et al. 1986). With remarkable affinity for heavy metal ions, it can efficiently remove them from dilute solutions. It works like a commercial ion-exchange resin. It can be packed into columns and can be recycled or reused without loss in its efficiency. It can be employed in the treatment of metal-bearing wastewater, ground water and drinking water as well as in the recovery of precious metals. AMT-BIOCLAIM™, developed by Advanced Mineral Technology Inc. (AMT), is a bacterial biosorbent that uses the biomass of *Bacillus subtilis* (Brierley et al. 1986; Brierley 1990). It can be used for both wastewater treatment and metal recovery with very high removal

efficiency. BIO-FIX, which was proposed by U.S. Bureau of Mines, consists of the biomass of cyanobacterium (*Spirulina* sp.), yeast, algae and plants like *Lemna* sp. and *Sphagnum* sp. immobilized in artificial polymers, such as polysulphone, polyethylene and polypropylene, to form porous beads (Michalak et al. 2013).

6.4 Cyanobacteria in Bioremediation of Heavy Metals

Over the years, cyanobacteria have attracted considerable attention as potential agents of bioremediation for the control of pollution caused by various pollutants, including heavy metals (Lem and Glick 1985; Subramanian and Uma 1996, 2001; Kuritz 1999; Radwan and Al-Hasan 2000; Gupta et al. 2013). They have the capacity to degrade a wide range of organic pollutants, such as pesticides (Megharaj et al. 1994; Mansy and El-Bestawy 2002; El-Bestawy et al. 2007), hydrocarbons or crude oil (Al-Hasan et al. 1998, 2001; Sorkhoh et al. 1992), phenol and catechol (Ellis 1977; Shashirekha et al. 1997; Wurster et al. 2003), naphthalene (Cerniglia et al. 1980a, b), phenanthrene (Narro et al. 1992) and synthetic dyes (Parikh and Madamwar 2005). They have been reported to be effective biological agents for the transformation and removal of heavy metals (Bender et al. 1995; Faisal et al. 2005; Lefebvre et al. 2007). They can be employed as a suitable and low-cost agents in biological wastewater treatment systems for the treatment of domestic and industrial wastewater (El-Bestawy 2008), dairy wastewater (Lincoln et al. 1996), fish farm effluents (Duma et al. 1998) and wastewater containing excess amount inorganic nutrients, such as nitrate and phosphate, which may lead to the eutrophication of water bodies (Hu et al. 2000; Ogbonna et al. 2000; Chevalier et al. 2000; Lodi et al. 2003; De-Bashana and Bashana 2004). Moreover, they can be used in bioremediation of oil spills and oil-polluted sites (Sorkhoh et al. 1995; Radwan and Al-Hasan 2001; Raghukumar et al. 2001; Cohen 2002).

The studies on cyanobacteria–metal interactions received increased attention in view of potential applications in bioremoval of heavy metals from polluted water or wastewater (Wilde and Benemann 1993; Liehr et al. 1994; Bender et al. 1994; Mehta and Gaur 2005; Roeselers et al. 2008). Aquatic bodies are favourable habitats of cyanobacteria, supporting their rich growth and biodiversity. The aquatic ecosystems, where cyanobacteria constitute important primary producers, are major sinks of heavy metals released as a result of various anthropogenic activities. Cyanobacteria have developed several effective mechanisms for tolerating heavy metals which enable resistant species/strains to grow and survive under high levels of heavy metals (Verma and Singh 1995; Faisal et al. 2005). Moreover, they are abundant and can be dominant organisms in certain metal-contaminated aquatic bodies and soil (Say and Whitton 1980; Whitton 1980; Whitton and Shehata 1982). Due to their remarkable ability to sequester metals and to their abundance in natural environments, particularly water and soil, they contribute significantly to the heavy metal sequestration in nature. Known mechanisms of metal resistance in cyanobacteria include (1) exogenous (extracellular) metal chelation by exopolysaccharides (EPS)

of mucilaginous sheath or capsule (Fiore and Trevors 1994; Ozturk and Aslim 2008), (2) transformation or reduction of toxic form of a metal to relatively less toxic or non-toxic form (Garnham and Green 1995; Faisal et al. 2005; Lefebvre et al. 2007), for instance, toxic Cr^{6+} to less toxic Cr^{3+}, (3) energy-dependent metal efflux (Verma and Singh 1991, 1995), and (4) endogenous (intracellular) metal chelation or sequestration by metallothioneins (MTs), a group of inducible low molecular weight cysteine-rich metal-binding proteins (Turner and Robinson 1995; Olafson 1986), and by polyphosphate granules, the linear polymers of inorganic phosphate synthesized as cellular inclusions under the phosphorus surplus (Jensen et al. 1982; Pettersson et al. 1985). Cyanobacteria take up heavy metals from the ambient environment or medium in two phases: initial rapid binding of metal cations to the negatively charged groups on the cell surface, a metabolism-independent passive process, followed by relatively slower metabolism-dependent active intracellular import or transport of metal cations (Pant et al. 1992; Pandey and Singh 1993; Fiore and Trevors 1994). The former process is referred to as adsorption or biosorption, while the latter one is called uptake or bioaccumulation. Factors, such as cell population or density, physiological state of cells, initial metal concentration, pH, temperature, light intensity, membrane potential of target cells, presence of co-ions, available nutrients, salinity and contact time influence the metal uptake or biosorption in cyanobacteria (Verma and Singh 1990; Singh et al. 1992; Fiore et al. 1998; Cain et al. 2008). A large number of cyanobacteria are known to possess heavy metal removal capability (Table 6.1). There is considerable potential in the use of such cyanobacteria in the detoxification heavy metal-bearing industrial effluents and wastewater (Wilde and Benemann 1993; Bender et al. 1994; Mehta and Gaur 2005). Like other microbes, the metal sorption or removal capacity of cyanobacteria varies with the species/strains and the type or properties of metal ions.

Cyanobacteria are promising biosorbents for heavy metal bioremediation. The justifiable and relevant merits, in addition to heavy metal tolerance and removal, include their ubiquity, rapid growth rate, simple growth requirements, production of copious amount of EPS and amenability to controlled laboratory or mass culture. Furthermore, due to their photoautotrophic nature and the ability of some species to fix atmospheric nitrogen, the growth and maintenance of cyanobacteria is less expensive than those of other microbes. Both living (De Philippis et al. 2003; Tien et al. 2005; De Philippis et al. 2007) and non-living (Corder and Reeves 1994; Tien et al. 2005; Gupta and Rastogi 2008; Aneja et al. 2010) biomass of cyanobacteria can be utilized for the biosorption of heavy metals. The mass culture of cyanobacteria for commercial and bioremediation applications can be grown in both open-culture and highly controlled closed-culture systems. EPS of mucilaginous sheaths or capsules contribute significantly to the heavy metal sorption or sequestration in cyanobacteria. Therefore, EPS-producing cyanobacteria have attracted increased attention due to their immense potential in metal sorption (De Philippis et al. 2003; De Philippis and Vincenzini 1998; Parker et al. 2000; Raungsomboon et al. 2006; Paperi et al. 2006; De Philippis et al. 2007; Micheletti et al. 2008). EPS-containing sheaths/capsules act as buffer zone between cyanobacterial cells and ambient physico-chemical environment and contribute to desiccation tolerance, UV tolerance, formation of

Table 6.1 Cyanobacteria with heavy metal removal capability

Cyanobacteria	Heavy metals	References
Synechococcus PCC7942 and PCC6301	Cu, Pb, Ni, Cd, Cr	Gardea-Torresdey et al. (1998); Garnham and Green (1995)
Synechocystis sp. PCC6803	Cu	Kumar et al. (2014)
Anacystis nidulans	Ni, Zn, Cd	Singh and Yadava (1985); Awasthi and Rai (2004)
Aphanothece halophytica	Hg, As, Cd	Laloknam et al. (2009)
Aphanothece flocculosa	Hg	Cain et al. (2008)
Chroococcus paris	Cd, Cu, Zn	Les and Walker (1984)
Cyanothece strain ET5 and 16Som2	Cu, Cr	Micheletti et al. (2008)
Gloeocapsa gelatinosa	Pb	Raungsomboon et al. (2006)
Microcystis aeruginosa	Cu	Tien et al. (2005)
Phormidium sp.	Pb, Cu, Cd, Zn, Ni	Wang et al. (1998)
Phormidium sp.NTMS02	Pb, Cr	Kumar et al. (2011); Rajeshwari et al. (2012)
Oscillatoria sp.NTMS01	Pb, Cr	Kumar et al. (2011); Rajeshwari et al. (2012)
Oscillatoria laete-virens	Cr, Pb	Miranda et al. (2012a, b)
Oscillatoria trichoides	Cr	Miranda et al. (2012a)
Spirulina sp.	Pb, Zn	Aneja et al. (2010)
Spirulina platensis	Hg, Cu, Cd	Cain et al. (2008); Johnson and Subert (1986); Fang et al. (2011)
Anabaena variabilis	Cr, Zn, Cu	Garnham and Green (1995); El-Bestawy (2008)
Anabaena oryzae	Zn, Cu	El-Bestawy (2008)
Anabaena cylindrica	Cu, Ni	Tien et al. (2005); Corder and Reeves (1994); Campbell and Smith (1986)
Anabaena spiroides	Cu	Tien et al. (2005)
Anabaena flos-aquae	Ni	Corder and Reeves (1994)
Nostoc PCC7936	Cu, Cr, Zn, Ni	Micheletti et al. (2008), De Philippis et al. (2007); De Philippis et al. (2003)
Nostoc calcicola	Cu, Hg	Singh et al. (1989, 1992); Pandey et al. (1992); Pandey and Singh (1993)
Nostoc muscorum	Cu, Zn, Pb, Cd	Hazarika et al. (2015); Goswami et al. (2015)
Cyanospira capsulata	Cu, Cr, Zn, Ni	De Philippis et al. (2003); Paperi et al. (2006); De Philippis et al. (2007); Micheletti et al. (2008)
Aulosira fertilissima	Pb, Cu, Cd, Zn, Ni	Singh et al. (2007)
Tolypothrix ceytonica	Zn, Cu	El-Bestawy (2008)

biofilms, adherence to the substratum and gliding motility in cyanobacteria (De Philippis and Vincenzini 1998; Pereira et al. 2009). In addition to their use as metal biosorbents in wastewater treatment, these biopolymers have various industrial or biotechnological applications (De Philippis and Vincenzini 1998; De Philippis et al. 2001). Cyanobacterial EPS are complex heteropolymers (heteropolysaccharides) of anionic nature, consisting of ten different monosaccharides belonging to hexoses

(glucose, galactose and mannose), pentoses (ribose, xylose and arabinose), deoxyhexoses (fucose and rhamnose) and acidic hexoses (glucuronic acid and galacturonic acid) (De Philippis and Vincenzini 1998). The presence of acidic sugars—glucuronic acid and galacturonic acid accounts for the anionic (negatively charged) nature of EPS due to which EPS have high affinity for metal cations. In some cases, EPS are released into surrounding environment and are referred to as released polysaccharides (RPS). Several major and minor factors controlling the production of EPS in cyanobacteria are known. These include nutrients, light intensity, temperature, salinity, aeration, pH and culture age (growth phase) (Pereira et al. 2009).

Immobilization, the technique of entrapment or encapsulation of cells or biomolecules in a suitable polymer matrix, of whole microbial cells has attracted considerable attention particularly because of their potential for industrial applications. Immobilization of cyanobacteria and other microalgae offers several advantages over freely suspended (non-immobilized) cells in heavy metal removal (Nakajima et al. 1982; Rai and Singh et al. 1989; Wilkinson et al. 1990; Mallick and Rai 1993, 1994). These include (1) higher metal uptake rate or enhanced efficiency in metal removal, (2) better mechanical stability and strength, (3) resistance to microbial degradation, (4) improvement of functional longevity, (5) packing of spherical immobilized biomass beads in biosorption columns with great ease, (6) no washout of cells, (7) regeneration and reuse of the biomass after the desorption or elution of metals, (8) easier solid–liquid separation and (9) ease of harvesting. Additionally, the use of immobilized cyanobacteria can offer significant advantages in bioreactors due to better operational stability (Karel et al. 1985). In wastewater treatment operation, the employment of immobilized cyanobacterial cells can evade the problem of cell harvesting and separation of biomass from the treated water (de la Noüe and de Pauw 1988; Brouers et al. 1989; Mallick 2002). Several natural (e.g. alginate, agar, agarose, carrageenan, chitosan) and synthetic (e.g. polysulfone and epoxy resins, polyurethane, acrylamide) polymers are used as immobilization matrices (Blanco et al. 1999; Mallick 2002). Several studies have demonstrated the superiority of immobilized cells over non-immobilized free cells in heavy metal uptake and removal in cyanobacteria, such as *Anabaena doliolum* (Rai and Mallick 1992; Mallick and Rai 1994), *Phormidium laminosum* (Blanco et al. 1999), *Phormidium* sp. (Kumar et al. 2011; Rajeshwari et al. 2012), *Oscillatoria* sp. (Kumar et al. 2011; Rajeshwari et al. 2012), *Nostoc calcicola* (Singh et al. 1989) and *Anacystis nidulans* (Awasthi and Rai 2004).

6.5 Conclusions

Due to various merits and advantages, bioremediation has emerged as an attractive alternative to the existing conventional clean-up technologies that has tremendous application in environmental management and pollution control. Although the process of heavy metal biosorption and microbial biosorbents have been extensively investigated in laboratories, the development of suitable biosorption technologies

and their implementation in various sectors releasing heavy metals have not gained desired momentum. However, a limited number of companies have developed and commercialized microbe-based bioremediation technologies. Evaluation and development of biosorbent materials from microbial biomass is a rapidly developing area of research. Removal as well as recovery of metals, including heavy metals, from metal-contaminated industrial effluents and wastewater exploiting the metal accumulation or biosorption capacity of a wide range of organisms, including cyanobacteria, has attracted increased attention. Cyanobacteria are a fascinating and unique group of organisms with remarkable adaptability, ubiquity and diversity. Although cyanobacteria growing in aquatic and terrestrial habitats are easily accessible, screening and selection of the promising cyanobacterial species/strains with high metal sorption or removal capacity is a challenging task. The promising species/strains can be suitable candidates for the development of new and efficient biosorbents which can be packed in biosorption columns. In order to fully utilize the potential of cyanobacteria in bioremediation of heavy metals, efforts should be directed towards screening and selection of promising species/strains, development of more efficient biosorption columns with better performance, low-cost immobilization and mass culture techniques, and genetic manipulation of cyanobacteria for the enhancement of metal biosorption or uptake capacity and over expression of genes encoding metal-binding proteins and surface-bound chemical groups.

Acknowledgements The author wishes to thank Head (Department of Botany) and Principal, Govt. Post-Graduate College, Rishikesh for providing required facilities. Financial assistance from UGC, New Delhi and UCOST, Dehradun is gratefully acknowledged.

References

Abed RMM, Dobretsov S, Sudesh K (2009) Applications of cyanobacteria in biotechnology. J Appl Microbiol 106:1–12

Al-Hasan RH, Al-Bader DA, Sorkhoh NA, Radwan SS (1998) Evidence for n-alkane consumption and oxidation by filamentous cyanobacteria from oil-contaminated coasts of the Arabian Gulf. Mar Biol 130:521–527

Al-Hasan RH, Khanafer M, Eliyas M, Radwan SS (2001) Hydrocarbon accumulation by picocyanobacteria from the Arabian Gulf. J Appl Microbiol 91(3):533–540

Aneja RK, Chaudhary G, Ahluwalia SS, Goyal D (2010) Biosorption of Pb^{2+} and Zn^{2+} by non-living biomass of *Spirulina* sp. Indian J Microbiol 50(4):438–442

Awasthi M, Rai LC (2004) Adsorption of nickel, zinc and cadmium by immobilized green algae and cyanobacteria: a comparative study. Ann Microbiol 54(3):257–267

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27:355–384

Bencko V (1987) Arsenic. In: Fishbein L, Furst A, Mehlman MA (eds) Genotoxic and carcinogenic metals: environmental and occupational occurrence and exposure. Princeton Scientific Publishing, Princeton, NJ, pp 1–30

Bender J, Rodriguez-Eaton S, Ekanemesang UM, Phillips P (1994) Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. Appl Environ Microbiol 60:2311–2315

Bender J, Lee RF, Phillips PC (1995) Uptake and transformation of metals and metalloids by microbial mats and their use in bioremediation. J Ind Microbiol 14:113–118
Blanco A, Sanz B, Llama MJ, Serra JL (1999) Biosorption of heavy metals to immobilized *Phormidium laminosum* biomass. J Biotechnol 69:227–240
Brady D, Duncan JR (1994) Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 4:149–154
Brierley JA (1990) Production and application of a *Bacillus*-based product for use in metals biosorption. In: Volesky B (ed) Biosorption of heavy metals. CRC Press, Boca Raton, pp 305–311
Brierley JA, Brierley CA, Goyak GM (1986) AMT-BIOCLAIM: a new wastewater treatment and metal recovery technology. In: Lawrence RW, Branion RMR, Ebner HG (eds) Fundamental and applied biohydrometallurgy. Elsevier, Amsterdam, pp 291–308
Brouers M, Dejong H, Shi DJ, Hall DO (1989) Immobilized cells: an appraisal of the methods and applications of cell immobilization techniques. In: Cresswell RC, Rees TAV, Shah N (eds) Algal and cyanobacterial biotechnology. Longman Scientific and Technical Publication, New York, pp 272–290
Cain A, Vannela R, Woo LK (2008) Cyanobacteria as a biosorbents for mercuric ion. Bioresour Technol 99:6578–6586
Campbell PM, Smith GD (1986) Transport and accumulation of nickel ions in the cyanobacterium *Anabaena cylindrica*. Arch Biochem Biophys 244:470–477
Carr NG, Whitton BA (1982) The biology of cyanobacteria, Botanical monographs, vol 19. Blackwell Scientific Publications, Oxford
Castenholz RW, Waterbury JB (1989) Oxygenic photosynthetic bacteria. Group I cyanobacteria. In: Stanley JT, Bryant MP, Pfennig N (eds) Bergey's manual of systematic bacteriology, vol 3. Williams and Wilkins, Baltimore, MD, pp 1710–1798
Cerniglia CE, Gibson DT, Van Baalen C (1980a) Oxidation of naphthalene by cyanobacteria and microalgae. J Gen Microbiol 116:495–500
Cerniglia CE, Van Baalen C, Gibson DT (1980b) Metabolism of naphthalene by the cyanobacterium *Oscillatoria* sp., strain JCM. J Gen Microbiol 116:485–494
Chakarborty N, Banerjee A, Lahiri S, Panda A, Ghosh AN, Pal R (2009) Biorecovery of gold using cyanobacteria and an eukaryotic alga with special reference to nanogold formation-a novel phenomenon. J Appl Phycol 21:145–152
Chevalier P, Proulx D, Lessard P, Vincent WF, de la Noüe J (2000) Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment. J Appl Phycol 12:105–112
Chirwa EMN, Wang YT (1997) Biological reduction of hexavalent chromium by *Pseudomonas fuorescens* LB 300 in a fixed-film reactor. J Environ Eng 123:760–766
Cohen Y (2002) Bioremediation of oil by marine microbial mats. Int Microbiol 5:189–193
Corder SL, Reeves M (1994) Biosorption of nickel in complex aqueous waste streams by cyanobacteria. Appl Biochem Biotechnol 45(46):847–859
Costa M, Klein C (2006) Toxicity and carcinogenicity of chromium compounds in humans. Crit Rev Toxicol 36:155–163
Darnall DW, Greene B, Henzl MT, Hosea JM, McPherson RA, Sneddon J, Alexander MD (1986) Selective recovery of gold and other metal ions from algal biomass. Environ Sci Technol 20:206–208
de la Noüe J, de Pauw N (1988) The potential of microalgal biotechnology: a review of production and use of microalgae. Biotechnol Adv 6:725–770
De Philippis R, Paperi R, Sili C (2007) Heavy metal sorption by released polysaccharides and whole cultures of two exopolysaccharide-producing cyanobacteria. Biodegradation 18:181–187
De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–175
De Philippis R, Sili C, Paperi R, Vincenzini M (2001) Exopolysaccharide-producing cyanobacteria and their possible exploitation. J Appl Phycol 13:293–299
De Philippis R, Paperi R, Sili C, Vincenzini M (2003) Assessment of metal removal capability of two capsulated cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936. J Appl Phycol 15:155–161

De-Bashana LE, Bashana Y (2004) Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). Water Res 38:4222–4246

Duffus JH (2002) "Heavy metals"-a meaningless term? Pure Appl Chem 74(5):793–807

Duma A, Lalibertk G, Lessard P, de la Noiiea VJ (1998) Biotreatment of fish farm effluents using the cyanobacterium *Phormidium bohneri*. Aqua Eng 17:57–68

Duruibe JO, Ogwuegbu MOC, Egwurugwu JN (2007) Heavy metal pollution and human biotoxic effects. Int J Phys Sci 2(5):112–118

Ehling-Schulz M, Scherer S (1999) UV protection in cyanobacteria. Eur J Phycol 34:329–338

El-Bestawy E (2008) Treatment of mixed industrial wastewater using cyanobacteria. J Ind Microbiol Biotechnol 35:1503–1516

El-Bestawy E, Abd El-Salam AZ, Abd El-Rahman HM (2007) Potential use of environmental cyanobacterial species in bioremediation of lindane-contaminated effluents. Int Biodeter Biodegr 59(3):180–192

Ellis BE (1977) Degradation of phenolic compounds by freshwater algae. Plant Sci Lett 8:213–216

Faisal M, Hameed A, Hasnain S (2005) Chromium resistant bacteria and cyanobacteria: impact of Cr(VI) reduction potential and plant growth. J Ind Microbiol Biotechnol 32:615–621

Fan AM, Harding-Barlow I (1987) Chromium. In: Fishbein L, Furst A, Mehlman MA (eds) Genotoxic and carcinogenic metals: environmental and occupational occurrence and exposure. Princeton Scientific Publishing Co., Princeton, NJ, pp 87–126

Fang L, Zhou C, Cai P, Chen W, Rong X, Dai K, Liang W, Gu JD, Huang Q (2011) Binding characteristics of copper and cadmium by cyanobacterium *Spirulina platensis*. J Hazard Mater 190:810–815

Fiore MF, Trevors JT (1994) Cell composition and metal tolerance in cyanobacteria. Biometals 7:83–103

Fiore MF, Moon DH, Trevors JT (1998) Metal resistance and accumulation in cyanobacteria. In: Wong Y-S, Tam NFY (eds) Wastewater treatment with algae. Springer, Berlin, pp 111–124

Gardea-Torresdey JL, Arenas JL, Francisco NMC, Tiemann KJ, Webb R (1998) Ability of immobilized cyanobacteria to remove metal ions from solution and demonstration of the presence of metallothionein genes in various strains. J Hazard Sub Res 1:1–18

Garnham GW, Green M (1995) Chromate(VI) uptake by and interactions with cyanobacteria. J Ind Microbiol 14:247–251

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86:528–534

Goswami S, Diengdoh OL, Syiem MB, Pakshirajan K, Kiran MG (2015) Zn(II) and Cu(II) removal by *Nostoc muscorum*: a cyanobacterium isolated from a coal mining pit in Chiehruphi, Megahalaya, India. Can J Microbiol 61(3):209–215

Grimanis AP, Zafiropoulos D, Vassilaki R, Grimanis M (1978) Trace elements in the flesh and liver of two fish species from polluted and unpolluted areas in the Aegean Sea. Environ Sci Technol 12:723–726

Gupta VK, Rastogi A (2008) Biosorption of Pb(II) from aqueous solution by non-living algal biomass *Oedogonium* sp. and *Nostoc* sp.-a comparative study. Colloids Surf B Biointerfaces 64(2):170–178

Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H (2000) Microbial biosorbents: meeting challenges of heavy metal pollution in aqueous solutions. Curr Sci 78(8):967–973

Gupta V, Ratha SK, Sood A, Chaudhary V, Prasanna R (2013) New insights in to the biodiversity and applications of cyanobacteria (blue-green algae)- prospects and challenges. Algal Res 2(2):79–97

Hayes RB (1997) The carcinogenicity of metals in humans. Cancer Causes Control 8:371–385

Hazarika J, Pakshirajan K, Sinharoy A, Syiem MB (2015) Bioremoval of Cu(II), Zn(II), Pb(II) and Cd(II) by *Nostoc muscorum* isolated from coal mining site. J Appl Phycol 27:1525–1534

Hu Q, Westerhoff P, Vermaas W (2000) Removal of nitrate from groundwater by cyanobacteria: quantitative assessment of factors influencing nitrate uptake. Appl Environ Microbiol 66(1):133–139

Jensen TE, Baxter M, Rachlin JW, Jani V (1982) Uptake of heavy metals by *Plectonema boryanum* (Cyanophyceae) in to cellular components, especially polyphosphate bodies: an X-ray energy dispersive study. Environ Pollut 27:119–127

Johnson PE, Subert LE (1986) Accumulation of mercury and other elements by Spirulina (cyanophyceae). Nutr Rep Int 34:1063–1070
Kapoor A, Viraraghavan T (1995) Fungal biosorption- an alternative treatment option for heavy metal bearing wastewaters: a review. Bioresour Technol 53:195–206
Karel SF, Libicki SB, Robertson CR (1985) The immobilization of whole cells: engineering principles. Chem Eng Sci 40:1321–1353
Kumar MS, Rajeshwari K, Johnson S, Thajuddin N, Gunasekaran M (2011) Removal of Pb(II) by immobilized and free filaments of marine *Oscillatoria* sp.NTMS01 and *Phormidium* sp.NTMS02. Bull Environ Contam Toxicol 87:254–259
Kumar R, Singh K, Sarkar S, Sethi LN (2014) Accumulation of Cu by microalgae *Scendesmus obliquus* and *Synechocystis* sp. PCC6803. IOSR J Environ Sci Toxicol Food Technol 8(6):64–68
Kuritz T (1999) Cyanobacteria as agents for the control of pollution by pesticides and chlorinated organic compounds. J Appl Microbiol 85:186S–192S
Kuyucak N, Volesky B (1988) Biosorbents for recovery of metals from industrial solutions. Biotechnol Lett 10:137–142
Laloknam S, Sirisopana S, Phornphisutthimas S, Takabe T, Incharoensakdi A (2009) Removal of mercury, arsenic and cadmium in synthetic wastewater by cyanobacterium *Aphanothece halophytica*. NU Sci J 6:96–104
Lefebvre DD, Kelly D, Budd K (2007) Biotransformation of Hg(II) by cyanobacteria. Appl Environ Microbiol 73(1):243–249
Lem NW, Glick BR (1985) Biotechnological uses of cyanobacteria. Biotechnol Adv 3:195–208
Les A, Walker RW (1984) Toxicity and binding of copper, zinc and cadmium by blue-green alga, *Chroococcus paris*. Water Air Soil Pollut 23:129–139
Liehr SK, Chen HJ, Lin SH (1994) Metal removal by algal biofilms. Water Sci Technol 30:59–68
Lincoln EP, Wilkie AC, French BT (1996) Cyanobacterial process for renovating dairy wastewater. Bioengineering 10:63–68
Lloyd JR (2003) Microbial reduction of metals and radionuclides. FEMS Microbiol Rev 27:411–425
Lodi A, Binaghi L, Solisio C, Converti A, Del Borghi M (2003) Nitrate and phosphate removal by *Spirulina platensis*. J Ind Microbiol Biotechnol 30:656–660
Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15:377–390
Mallick N, Rai LC (1993) Influence of culture density, pH, organic acids and divalent cations on the removal of nutrients and metals by immobilized *Anabaena doliolum* and *Chlorella vulgaris*. World J Microbiol Biotechnol 9:196–201
Mallick N, Rai LC (1994) Removal of inorganic ions from wastewaters by immobilized microalgae. World J Microbiol Biotechnol 10:439–443
Mansy AH, El-Bestawy E (2002) Toxicity and biodegradation of fluometuron by selected members of cyanobacteria. World J Microbiol Biotechnol 18:125–131
McEldowney S, Hardman DJ, Waite S (1993) Pollution: ecology and biotreatment. Longman Scientific & Technical, Harlow
Megharaj M, Madhavi DR, Sreenivasulu C, Umamaheswari A, Venkateswarlu K (1994) Biodegradation of methyl parathion by soil isolates of microalgae and cyanobacteria. Bull Environ Contam Toxicol 53:292–297
Mehta SK, Gaur GP (2005) Use of algae for removing heavy metal ions from wastewater: progress and prospects. Crit Rev Biotechnol 25:113–152
Michalak I, Chojnacka K, Witek-Krowiak A (2013) State of the art for the biosorption process-a review. Appl Biochem Biotechnol 170:1389–1416
Micheletti E, Colica G, Viti C, Tamagnini P, De Philippis R (2008) Selectivity in heavy metal removal by exopolysaccharide-producing cyanobacteria. J Appl Microbiol 105:88–94
Miranda J, Krishnakumar G, Gonsalves R (2012a) Cr^{6+} bioremediation efficiency of *Oscillatoria laete-virens* (Crouan & Grouan) Gomont and *Oscillatoria trichoides* Szafer: kinetics and equilibrium study. J Appl Phycol 24:1439–1454

Miranda J, Krishnakumar G, D'Silva A (2012b) Removal of Pb^{2+} from aqueous system by live *Oscillatoria laete-virens* (Crouan and Crouan) Gomont isolated from industrial effluents. World J Microbiol Biotechnol 28:3053–3065

Murray RGE (1989) The higher taxa or a place for everything …….? In: Staley JT, Bryant M P, Pfennig N (eds) Bergey's manual of systematic bacteriology, vol. 3. Williams and Wilkins, Baltimore, MD, pp 1631–1634

Nakajima A, Horikoshi T, Sakaguchi T (1982) Recovery of uranium by immobilized microorganisms. Eur J Appl Microbiol Biotechnol 16:88–91

Narro ML, Cerniglia CE, Van Baalen C, Gibson DT (1992) Metabolism of phenanthrene by the marine cyanobacterium *Agmenellum quadriplicatum* PR-6. Appl Environ Microbiol 58:1351–1359

Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750

Nriagu JO, Pacyna JM (1988) Quantitative assessment of worldwide contamination of air, water and soils with trace metals. Nature 333:134–139

Ogbonna JC, Yoshizawa H, Tanaka H (2000) Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. J Appl Phycol 12:277–284

Olafson RW (1986) Physiological and chemical characterization of cyanobacterial metallothioneins. Environ Health Perspect 65:71–75

Oren A (2004) A proposal for further integration of the cyanobacteria under the bacteriological code. Int J Syst Evol Microbiol 54:1895–1902

Ozturk S, Aslim B (2008) Relationship between chromium (VI) resistance and extracellular polymeric substances (EPS) concentration by some cyanobacterial isolates. Environ Sci Pollut Res 15:478–480

Pandey PK, Singh SP (1993) Hg^{2+} uptake in a cyanobacterium. Curr Microbiol 26:155–159

Pandey PK, Singh CB, Singh SP (1992) Cu uptake in a cyanobacterium: fate of selected photochemical reactions. Curr Microbiol 24:35–39

Pandey VD, Gupta RK, Singh SK (2007) Cyanobacteria as a source of pharmaceutical compounds. In: Gupta RK, Pandey VD (eds) Advances in applied phycology. Daya Publishing House, New Delhi, pp 250–260

Pant A, Srivastava SC, Singh SP (1992) Methyl mercury uptake by free and immobilized cyanobacterium. Biometals 5:229–234

Paperi R, Micheletti E, De Philippis R (2006) Optimization of copper sorbing-desorbing cycles with confined cultures of the exopolysaccharide-producing cyanobacterium *Cyanospira capsulata*. J Appl Microbiol 101:1351–1356

Parikh A, Madamwar D (2005) Textile dye decolorization using cyanobacteria. Biotechnol Lett 27:323–326

Parker DL, Mihalick JE, Plude JL, Plude MJ, Clark TP, Egan L, Flom JJ, Rai LC, Kumar HD (2000) Sorption of metals by extracellular polymers from the cyanobacterium *Microcystis aeruginosa* f. *flos-aquae* strain C3-40. J Appl Phycol 12:219–224

Patterson GML (1996) Biotechnological applications of cyanobacteria. J Sci Ind Res 55:669–684

Pauls J, Rydzynski K, Dziubaltowska E, Wyszynska K, Natarajan AT, Nilsson R (2003) Genotoxic effects of occupational exposure to lead and cadmium. Mutat Res 540(1):19–28

Pereira S, Zille A, Micheletti E, Moradas-Ferreira P, De Philippis R, Tamagnini P (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941

Pettersson A, Kunst L, Bergman B, Romans GM (1985) Accumulation of aluminium by *Anabaena cylindrica* in to polyphosphate granules and cell walls: an X-ray energy-dispersive microanalysis study. J Gen Microbiol 131:2545–2548

Potts M (1999) Mechanisms of desiccation tolerance in cyanobacteria. Eur J Phycol 34:319–328

Radwan SS, Al-Hasan RH (2000) Oil pollution and cyanobacteria. In: BA W, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 307–319

Radwan SS, Al-Hasan RH (2001) Potential application of coastal biofilm-coated gravel particles for treating oily waste. Aquat Microbial Ecol 23:113–117

Raghukumar C, Vipparty V, David JJ, Chandramohan D (2001) Degradation of crude oil by marine cyanobacteria. Appl Microbiol Biotechnol 57:433–436

Rai LC, Mallick N (1992) Removal and assessment of toxicity of Cu and Fe to *Anabaena doliolum* and *Chlorella vulgaris* using free and immobilized cells. World J Microbiol Biotechnol 8:110–114

Rajeshwari K, Kumar MS, Thajuddin N (2012) Adsorption isotherms for Cr(VI) by two immobilized marine cyanobacteria. Ann Microbiol 62:241–246

Rao KR, Rashmi K, Latha JNL, Mohan PM (2005) Bioremediation of toxic metal ions using biomass of *Aspergillus fumigatus* from fermentative waste. Indian J Biotechnol 4:139–143

Raungsomboon S, Chidthaisong A, Harvey NW (2006) Production, composition and Pb^{2+} adsorption characteristics of capsular polysaccharides extracted from a cyanobacterium *Gloeocapsa gelatinosa*. Water Res 40:3759–3766

Rayms-Keller A, Olson KE, McGaw M, Oray C, Carlson JO, Beaty BJ (1998) Effect of heavy metals on *Aedes aegypti* (Diptera:Culicidea) larvae. Ecotoxicol Environ Saf 39:41–47

Robinson NJ, Rutherford JC, Pocock MR, Cavet JS (2000) Metal metabolism and toxicity: repetitive DNA. In: BA W, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 443–463

Roeselers G, van Loosdrecht MCM, Muyzer G (2008) Phototrophic biofilms and their potential applications. J Appl Phycol 20:227–235

Say PJ, Whitton BA (1980) Change in flora down a stream showing a zinc gradient. Hydrobiologia 76:255–262

Schopf JW (2000) The fossil record: tracing the roots of the cyanobacterial lineage. In: BA W, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 13–35

Shashirekha S, Uma L, Subramanian G (1997) Phenol degradation by the marine cyanobacterium *Phormidium valderianum* BDU-30501. J Ind Microbiol Biotechnol 19:130–133

Singh SP, Yadava V (1985) Cadmium uptake in *Anacystis nidulans*: effect of modifying factors. J Gen Appl Microbiol 31:39–48

Singh SP, Verma SK, Singh RK, Pandey PK (1989) Copper uptake by free and immobilized cyanobacterium. FEMS Microbiol Lett 60:193–196

Singh SP, Singh RK, Pandey PK, Pant A (1992) Factors regulating copper uptake in free and immobilized cyanobacterium. Folia Microbiol 37(4):315–320

Singh A, Mehta SK, Gaur JP (2007) Removal of heavy metals from aqueous solution by common freshwater filamentous algae. World J Microbiol Biotechnol 23:1115–1120

Sorahan T, Waterhouse JAH (1985) Cancer of the prostrate among nickel-cadmium battery workers. Lancet 325(8426):415–474

Sorkhoh NA, Al-Hasan RH, Radwan SS, Hopner T (1992) Self cleaning of the Gulf. Nature (London) 359:109

Sorkhoh NA, Al-Hasan RH, Khanafer M, Radwan SS (1995) Establishment of oil-degrading bacteria associated with cyanobacteria in oil-polluted soil. J Appl Bacteriol 78:194–199

Stanier RY, Cohen-Bazire G (1977) Phototrophic prokaryotes: the cyanobacteria. Annu Rev Microbiol 31:225–274

Stanier RY, Sistrom WR, Hansen TA, Whitton BA, Castenholz RW, Pfennig N, Gorlenko VN, Kondratieva EN, Eimhjellen KE, Whittenbury R, Gherma RL, Truper HG (1978) Proposal to place nomenclature of cyanobacteria (blue-green algae) under rules of international code of nomenclature of bacteria. Int J Syst Bacteriol 28:335–336

Subramanian G, Uma L (1996) Cyanobacteria in pollution control. J Sci Ind Res 55:685–692

Subramanian G, Uma L (2001) Potential applications of cyanobacteria in environmental biotechnology. In: Lee YK (ed) Kojma H. Springer, Photosynthetic microorganisms in environmental biotechnology, pp 41–49

Tandeau de Marsac N, Houmard J (1993) Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol Rev 104:119–190

Tien C-J, Sigee DC, White KN (2005) Copper adsorption kinetics of cultured algal cells and freshwater phytoplankton with emphasis on cell surface characteristics. J Appl Phycol 17:379–389
Turner JS, Robinson NJ (1995) Cyanobacterial metallothioneins: biochemistry and molecular genetics. J Ind Microbiol 14:119–125
Van Den Hoek C, Mann DG, Jahns HM (1995) Algae: an introduction to phycology. Cambridge University Press, Cambridge
Verma SK, Singh SP (1990) Factors regulating copper uptake in a cyanobacterium. Curr Microbiol 21:33–37
Verma SK, Singh HN (1991) Evidence for energy-dependent copper efflux as a mechanism of Cu^{2+} resistance in the cyanobacterium *Nostoc calcicola*. FEMS Microbiol Lett 84:291–294
Verma SK, Singh SP (1995) Multiple metal resistance in the cyanobacterium *Nostoc muscorum*. Bull Environ Contam Toxicol 54:614–619
Vieira RHSF, Volesky B (2000) Biosorption: a solution to pollution? Int Microbiol 3:17–24
Volesky B (1994) Advances in biosorption of metals: selection of biomass types. FEMS Microbiol Rev 14:291–302
Wang J, Chen C (2006) Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. Biotechnol Adv 24(5):427–451
Wang TC, Weissman JC, Ramesh G, Varadharajan R, Benemann JR (1998) Heavy metal binding and removal by *Phormidium*. Bull Environ Contam Toxicol 60:739–744
Waterbury JB, Watson SW, Guillard RRL, Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature 227:293–294
Weast RC (1984) CRC handbook of chemistry and physics, 64th edn. CRC, Boca Raton, FL
Whitton BA (1980) Zinc and plants in rivers and streams. In: de Nriagu JO (ed) Zinc in the environment: part II, health effects. Wiley, New York, pp 364–400
Whitton BA, Shehata FHA (1982) Influence of cobalt, nickel, copper and cadmium on the bluegreen alga *Anacystis nidulans*. Environ Pollut 27:275–281
Wilde EW, Benemann JR (1993) Bioremoval of heavy metals by the use of microalgae. Biotechnol Adv 11:781–812
Wilkinson SC, Goulding KH, Robinson PK (1990) Mercury removal by immobilized algae in batch culture systems. J Appl Phycol 2:223–230
Wurster M, Mundt S, Hammer E, Schauer F, Lindequist U (2003) Extracellular degradation of phenol by the cyanobacterium *Synechococcus* PCC 7002. J Appl Phycol 15:171–176
Zweig RD, Morton JD, Stewart MM (1999) Source water quality for aquaculture: a guide for assessment. The World Bank, Washington, DC

Chapter 7
Biochar Application in Management of Paddy Crop Production and Methane Mitigation

Chhatarpal Singh, Shashank Tiwari, Siddharth Boudh, and Jay Shankar Singh

Abstract Paddy agriculture is one of the major anthropogenic sources of methane (CH_4) emission at global level. A decrease in CH_4 release in the atmosphere from paddy fields can add significantly to the management of global warming and climate change. Biochar production and application in agriculture prepared from crop straw has been proposed as one of the effective countermeasure to mitigate the greenhouse gas emissions (GHGs) during farming. Biochar, a co-product of a controlled pyrolysis process, can be used as a tool to offset GHGs emissions and as a soil conditioner. Biochar application increased rice productivity, soil pH, soil organic carbon, total N but decreased soil bulk density in the long term. Recent studies have confirmed that the use of biochar in paddy agriculture has the capability to minimise the CH_4 production, but its essential mechanism has yet to be clarified. The additions of biochar to the agriculture soil showed higher CH_4 consumption because it improves soil aeration and porosity and enhances methanotrophs performance. However, further investigations are needed to evaluate the effect of biochar addition on net CH_4 emissions and consumptions, respectively, by methanogens and methanotrophs. Long-term experiments should be conducted to monitor any changes over the years on the influence of biochar amendments on soil–methanotrophs–paddy systems.

Keywords Biochar • GHGs • Methane • Methanotrophs • Paddy

C. Singh • S. Tiwari • S. Boudh
Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Raibarelly Road, Lucknow, Uttar Pradesh 226025, India

J.S. Singh (✉)
Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@bbau.ac.in

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_7

7.1 Introduction

Methane (CH_4) is one of the most widespread greenhouse gases (GHGs) emitted from paddy fields and other sources such as wetlands, ruminants, coal mines as well as anthropogenic activities such as leakage from natural gas systems and the raising of livestock. In the early nineteenth century, the atmospheric concentration of CH_4 was 700 ppb, but the current concentration is 1750 ppb and has shown a 1 % $year^{-1}$ increase rate over a century (IPCC 2001; Tiwari et al. 2015). The concentration of CH4 in the atmosphere is increasing due to discrepancy in CH_4 emanation and its removal (Singh 2010). The lifetime of CH_4 in the atmosphere is 8–12 years, but it is more efficient in trapping radiation and 23–30 times more potential than CO_2 (Tiwari et al. 2015). Global surface temperature has increased by 0.8 °C in the last 100 years and CH_4 also contributed to this phenomenon as a potent GHG (Hanson et al. 1996). Recent global estimates of CH_4 emission rates from wetland rice fields ranged from 20 to 100 Tg $year^{-1}$, which corresponds to 6–29 % of the total annual anthropogenic CH_4 emission (Neue 1993). According to Demisie and Zhang (2015) the processes of CH_4 emission are affected by soil texture, inorganic electron acceptors, soil physico-chemical properties and methanogenic population. CH_4 affects the chemistry and oxidation capacity of the atmosphere, e.g. by influencing concentrations of tropospheric ozone, hydroxyl radicals and carbon monoxide. The current burden of CH_4 in the atmosphere is approximately 4700 Tg $year^{-1}$ (Neue 1993). CH_4 is produced in flooded paddy soils by a group of bacteria designated as methanogens (also called marshy soil bacteria). The flooding rice fields restrict the oxygen supply to the soil, which may result in the anaerobic fermentation of soil organic matter and consequently release of sufficient amount of CH_4 to the atmosphere. From deeper layer of flooded soil CH_4 reaches to the atmosphere by diffusion, ebullition and through aerenchyma conduits of paddy plant. It is now well accepted that rice cultivation is the substantial source of CH_4 emissions therefore, there is need to management of flooded paddy agriculture to minimise the soil CH_4 emissions.

Keppler et al. (2006) demonstrated that significant amounts of CH_4 are produced from terrestrial plants and detached leaves. They assumed that living plants and plant litter produce 62–236 Tg $year^{-1}$ and 1–7 Tg $year^{-1}$ CH_4, respectively. Natural sources are accountable for about 30 % (up to160 Tg $year^{-1}$) of the CH_4 flux; however, the anthropogenic sources are responsible for contributing 70 % (up to 375 Tg $year^{-1}$) (Mer and Roger 2001). Soil amended with biochar produced less quantities of CH_4 than without biochar amendments. Biochar is a co-product of high concentration of carbon and silica which is produced by pyrolysis of biomass/organic material or plant residues under high temperature (400–500 °C) and low oxygen conditions (Lehmann 2007; Lehmann and Joseph 2009). It contains highly condensed aromatic compounds which are resistant to decomposition in soil and thus can effectively sequester the carbon. It is assumed that biochar application in agriculture may improve the soil fertility, crops yields, water holding capacity, degraded land restoration and support CH_4-assimilating microorganism, i.e. methanotrophs (Singh and Gupta 2016). Biochar used in agriculture soil as a soil conditioner and plant growth enhancer also increases microbial biomass in paddy ecosystem.

Impacts of biochar application on soil physico-chemical properties are widely known, while the research on agriculture productivity and CH_4 emission/consumption with reference to biochar application in paddy agriculture is scarce. Therefore, the objectives of this review are (1) to describe the impact of biochar on paddy productivity and CH_4 emission/consumption, (2) to assess the role of biochar amendment on soil microbial processes and biomass and (3) to discuss impact of biochar application on soil N dynamics.

7.2 What Is Biochar?

Biochar is a unique product, which enhances the plant-available nutrients and significantly improves the crop yield. Biochar is produced by pyrolysis of biomass or organic materials and this practice is termed as thermal degradation of biomass such as rice straw, grass, wood, agricultural wastes and manure (Wu et al. 2015). In addition, biochar can significantly improve soil properties by decreasing methane emission, soil bulk density; enhancing soil pH, organic carbon; increasing available nutrients; removing heavy metals and increasing number of methanotrophs, thus ultimately increasing crop yields (Milla et al. 2013). Biochar is fine-grained residue with a high carbon content and works as soil conditioner and carbon sequestrating agent in the soil (Johannes 2007; Gaunt and Johannes 2008; Peter 2007). As stated earlier, biochar enhances the crop yield and it is also indirectly involved in the mitigation of environmental pollution, such as reduction of GHGs. Therefore, most of the studies on biochar concentrated over large-scale production. (Peter 2007; Laird 2008; Johannes 2007; Ghoneim and Ebid 2013). Previously, it was reported that biochar increases the agriculture production and mitigates CH_4 emissions. However, biochar also increases the soil methanotrophic community structure and reduces the soil CH_4-generating bacteria (methanogens). Therefore, extensive work is required to assess the use of biochar and its impact to restore the methanotrophs niche in the disturbed paddy agriculture and its contribution to stabilise the atmospheric CH_4 concentration.

7.3 Biochar Production and Its Properties

Biochar production is a thermal degradation phenomenon of organic material and biomass, using a small-scale reactor and drum method at 400–500 °C with the residence time of up to 1 h. Table 7.1 presents different types of biochar produced from various sources (feedstock) (Gaunt and Johannes 2008; Peter 2007). Ca, Si, Al and K are common elements in biochar but C, N, H and S are also determined by a dry oxidation using an elemental analyser (Hmid et al. 2014). According to Gaunt and Johannes (2008) and Peter (2007) the performance of biochar in their original shapes can be detected by using grinders or sieves, including scanning electron microscopy (SEM) equipped with an energy-dispersive X-ray spectroscopy (EDX), Fourier

Table 7.1 Physico-chemical properties of feedstock for biochar production

Component	Woodchip Yargicoglu et al. (2015)	Grass Jouiada et al. (2015) and Mohammed et al. (2015)	Poultry litter Jindo et al. (2012)	Rice Husk Shackley et al. (2012)	Sugarcane Carriea et al. (2012)	Wheat straw Bruun et al. (2012), Mahinpeye et al. (2009), and Khan and Mubeen (2012)
Soil C (%)	74.5	–	71.47	–	–	–
Ash (%)	25.4	14.7	28.53	6.5	11.9–16.4	5.9
pH	7.88	6.1	23.596	6.6	–	6.76
EC (mS cm^{-1})	0.14	–	3.0	–	–	2770
CEC (c mol kg^{-1})	–	–	–	45–110	–	–
C (%)	51.9	42.5	38.6	41	60.4–65.3	43.7
N (%)	0.4	1.9	1.37	1.4	0.8–1.0	0.9
S (%)	–	5.3	–	0.1	25.4–15.1	0.283
Ca (ppm)	0.56	4.3 4	1.85	250	–	0.18
K (ppm)	0.21	64.80	0.99	2604	–	0.15
Mg (ppm)	0.04	2.3 4	0.19	827	–	–
Si mg kg^{-1}	–	7.44	–	5.8	–	0.18
P (ppm)	0.06	2.31	0.35	–	–	0.05

transform infrared spectroscopy (FTIR), volatile matter (VM), electrical conductivity (EC), total dissolved solids (TDS) analysis, water holding capacity (WHC) and heavy metal assessment. Peter (2007) and Milla et al. (2013) reported that the sample powder is sprinkled as a thin layer on an adhesive tape placed on the brass sample holder. Excess amounts of the sample are removed with a small manual air blower. The adhered sample is then coated with gold powder using a sputtering device, FTIR spectrometer identified the sample to determine the organic functional groups present for each biomass, especially carbons. Volatile matter in biochar is determined following the ASTM D 3175-07 standard test method. A Beckman Coulter SA 3100 BET analyser containing approximately 0.1000 g to 0.2000 g of each biochar sample is then used at a temperature of 50 °C for 60 min. Electrical conductivity and total dissolved solid analysis are theoretically the best measure to indicate the actual salinity level experienced by the plant root (Peter 2007). Hence, electrical conductivity and total dissolved solids are measured using a portable conductivity meter.

7.4 Biochar Types

Currently, varieties of feedstock are being used as raw material for the preparation of biochar at variable temperatures such as 250, 300, 400, 500, 600 and 700 °C. The composition of various nutrients of biochar varied during its preparation at variable temperatures. A variety of biochar from different feedstock has been presented in Fig. 7.1.

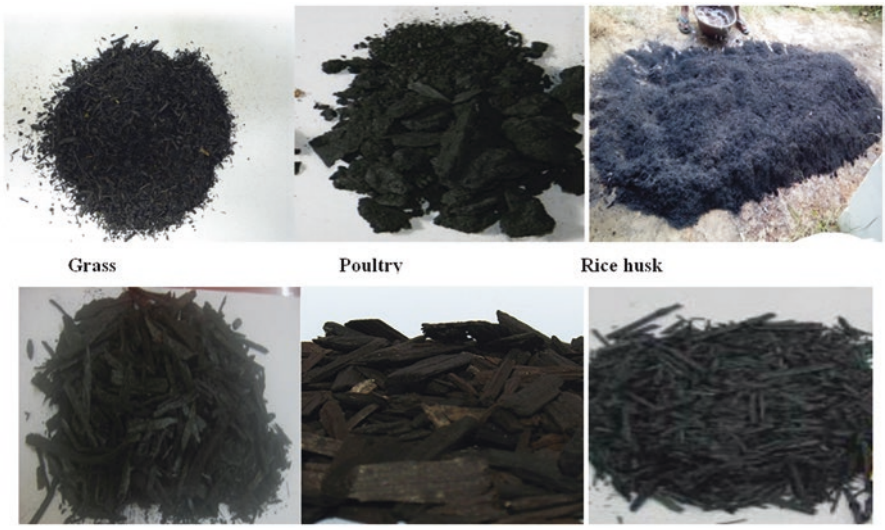


Fig. 7.1 Different feedstocks used for biochar production

7.4.1 Biochar Produced from Grass

Grass biochar is produced by a variety of grasses such as Switchgrass (*Panicum virgatum* L.), Sawgrass (*Cladium jamaicense*), etc. and has been declared as a model bioenergy crop for the production of biochar. These are preferred due to its high yield potential, low input requirements on marginal soils and potentially active in soil carbon sequestration and alleviation of GHGs (McLaughlin and Walsh 1998; Sadaka et al. 2014; Mukherjee and Lal 2013). The switchgrass has a gross calorific value between 18 and 19 MJ kg^{-1} as compared to hardwoods 20–21 MJ kg^{-1} (Sadaka et al. 2014). There were several barriers in the way of switchgrass to be used as the sole source of fuel in combustors such as high moisture and ash contents in biomass, which cause ignition and combustion problems. It has been observed that blending of biomass with coal would reduce flame stability problems and will also lead to significant reductions in methane emissions. Consequently, a multitude of studies has investigated about conversion of switchgrass to biochar for the safe and eco-friendly cultivation of agriculture crops (Sadaka et al. 2014).

7.4.2 Woodchips Biochar

Woodchips are a medium-sized solid material made by cutting, or chipping, larger pieces of wood. Today, woodchips are used as a raw material for the production of biochar. It has more carbon concentration as compared to other feedstock biochar

including the highest carbon sequestration potential. Woodchips feedstock produce a high quality biochar at 400–500 °C, its good residential time is 2–3 h. Woodchips absorb moisture at 15–20 °C, therefore it requires drying before the pyrolysis (Milla et al. 2013; Lai et al. 2013; Spokas et al. 2009). The *Camellia japonica* (Japanese Cedar) waste wood chips are used for biochar production by pyrolysis at either 290 °C or 700 °C and called biochar 290 (BC290) and biochar 700 (BC700), respectively (Lai et al. 2013). The percentage amount of C, N, H and available K contents have been found to be about 59.1 %, 0.35 %, 5.73 % and 0.78 g/kg for woodchips biochar 290 (BC290) and 83.0 %, 0.34 %, 2.57 % and 3.90 g/kg for BC700, respectively (Lai et al. 2013).

7.4.3 Rice Husk Biochar

Rice hulls (husk) are the coatings of seeds, or grains, of rice. The husk protects the seed during the growing season, because it is formed from hard materials, including silica, carbon, magnesium and phosphorus. Presently rice husk, used as a raw material for the production of biochar, improves the soil fertility and crop productivity. For making biochar, rice husk is put in a pyrolysis apparatus which consists of a stainless reactor of 500 mm length with a 15 cm inside diameter. The rice husk is then heated externally by an electric furnace (5000 W) to a temperature of 600 °C and it has more concentration of silica and carbon (Zhang and Liu 2012). The use of rice husk biochar in agriculture field in place of synthetic fertilisers is advantageous because the synthetic fertilisers generate many harmful effects such as reduction in microflora, crop yield, nutrient availability, water holding capacity, etc. The studies on cowpea, soybean and maize have also supported the application of biochar as a way to increase crop yields. In Asian region due to elevated production of rice, it is estimated that up to 560 and 112 million tons of rice straw and rice husks are produced, respectively. These residues may be a valuable resource for the production of biochar that may be used in agricultural applications (Masulili and Utomo 2010).

7.4.4 Poultry Litter Biochar

For the production of poultry litter biochar chicken manure (CM), the feedstocks used are wood feedstock, rice husk, plant residue, etc. (Songa and Guo 2012; Demirbas 2001). According to Songa and Guo (2012) CM is a solid waste material, resulting from chicken rearing and is being explored as a feedstock for biofuels and biochar. CM is a mixture of bedding materials of bird feather, hen's excreta and feed spills. These are pyrolysed by thermochemical conversion technology whereby organic materials are heated in the absence of oxygen. CM can be readily transformed into biochar, biofuel and syngas for the enhancing production of agricultural crop (Songa and Guo 2012; Kim et al. 2009).

7.4.5 *Sugarcane Bagasse Biochar*

Sugarcane industry produces several pyrolysable residues. These include bagasse (crushed cane stalks), cane trash (leaves and stalk tips removed during harvest) and filter cake, a sludge that is removed via filtration after the juice clarification step and bagasse used for many purposes such as biochar production, biofuels, burning purpose, etc. Currently, sugarcane bagasse is being used on large scale for the production of biochar. The raw material/feedstock should be dry in wet season because moisture content creates difficulties during pyrolysis; dry feedstock has a low residential time (1–2 h) for the production of biochar (Eykelbosh et al. 2014). Sugarcane biochar contains a high concentration of carbon, silica, magnesium, etc. and may play a significant role in agriculture field to enhance the crop production and as a conditioner for saline and degraded soil (Eykelbosh et al. 2014).

7.4.6 *Wheat Straw Biochar*

Wheat straw containing lignocelluloses biomass is the most abundant organic raw material and is being used widely for biochar production. Wheat straw is collected by a cutting machine and then shipped to the production plant and air-dried. Pyrolysis of wheat straw is performed in a vertical kiln at 350–550 °C, converting 35 % of the biomass to biochar. The biochar mass originally in a particulate form is ground to pass through a 2 mm sieve and mixed thoroughly to obtain a fine granular consistency that would mix more uniformly with the soil mass (Wu et al. 2013).

7.5 Impact of Biochar on Soil and Plant Growth

- Increases water holding capacity and reduces soil bulk density of the soil
- Enhances cation exchange capacity
- Improves fertiliser utilisation by reducing leaching from the root zone
- Retains minerals in plant available form
- Supports soil microbial life and biodiversity
- Plants resistance to diseases and pathogens
- Reduces soil CH_4 emission
- Increases soil methanotrophs population
- Improves soil carbon pool
- Increases nitrogen retention
- Promotes paddy root growth (Fig. 7.2)

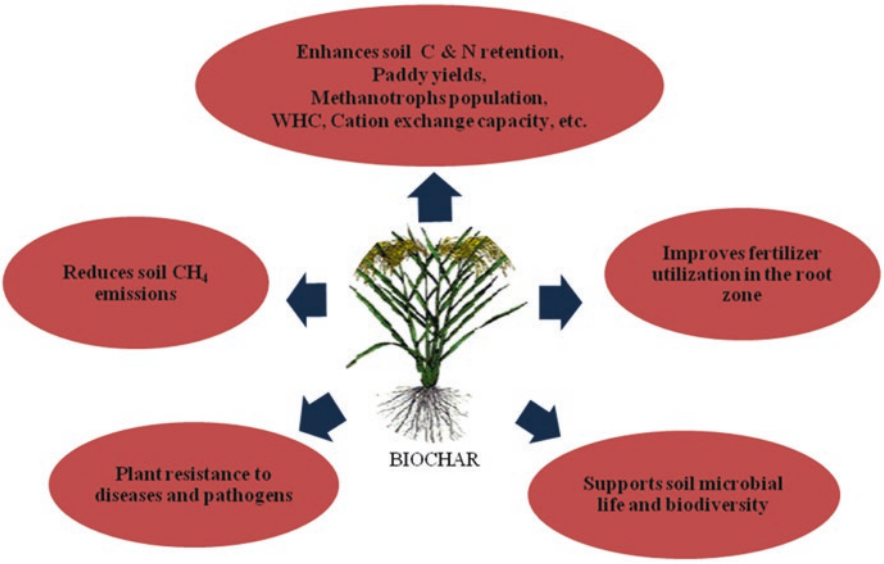


Fig. 7.2 Biochar applications in paddy field

7.6 Impact of Biochar on Crop Yields and Soil Properties

Biochar applications to increasing crop productivity by improving the physico-chemical and biological properties of the soil with variation in crop response. These impacts depend on the chemical and physical properties of the biochar, soil conditions and the crop type (Zwieten et al. 2010; Yamato et al. 2006). Zhanga et al. (2010a, b) found that biochar amendment at 10 t ha^{-1} and conventional N fertilisation at 300 kg ha^{-1} enhance the crop yield by 9 %, while only biochar amendment at 40 t ha^{-1} yields increased by12 %. However, the exact mechanism about the biochar effect on rice yield in presence or absence of fertiliser is still not known. Most of the previously reported field trials have been conducted mostly in tropical regions having relatively poor soils with the rain-fed crops (Zhanga et al. 2010a, b). Zhanga et al. (2010a, b) reported that biochar application increased rice yields by around 10 %. The biochar amendments can increase N availability to crops and that high level of soil organic C accumulation can enhance N efficiency and increase rice productivity in a long-term monitored rice paddy (Pan et al. 2009). This is of particular importance for world's rice agriculture as the farming has tremendous challenge of N pollution from overuse of N fertilisers (Zhanga et al. 2010a, b).

7.6.1 Paddy Productivity

Biochar amendment significantly impacts the crop yield including the improvement of root length, shoot biomass, panicle length, number of tiller per plan, rice yield, nutrient availability and carbon sequestration (Milla et al. 2013; Abdullah and Wu 2009; Meyer et al. 2011). However, Yang et al. (2015) reported that 2 ton ha^{-1} biochar application could increase the yield by 5–15 % and biochar of 4 ton ha^{-1} may increase the yield by about 20 %. The property of cation exchange capacity (CEC), pH and WHC of soils amended with biochar also increases (Yang et al. 2015).

7.6.2 Physico-chemical Properties of Soil

Biochar-amended soil shows the variation in many of its chemical properties, viz. pH, K, Ca, Mg, NH_4-N and NO_3-N as well as in the ratios of organic C, N and P (Jien and Wang 2013). They demonstrated that pH significantly increased from 7.41 to 9.26 with the application of biochar in the farming land. However, Prommer et al. (2014) reported that, after biochar amendment the soil pH and cation exchange capacity decreased slightly from a preliminary 7.5 to 7.4 (Table 7.2). The biochar-amended

Table 7.2 Physico-chemical properties of soil after amendment of biochar

Parameter	Unit	Control	Biochar
pH ($CaCl_2$)		7.5	7.4
$CaCO_3$	%	15.8	15.2
Humus	%	2.4	18.1
Total N	%	0.148	0.203
P (CAL)	mg kg^{-1}	49	84
P_{tot} (acid digest)	g kg^{-1}	5.46	5.54
Sand	%	18.3	Not determined
Silt	%	57.2	Not determined
Clay	%	24.5	Not determined
CEC	cmol kg^{-1}	22.5	20.8
Ca (CEC)	cmol kg^{-1}	20.7	18.2
Mg (CEC)	cmol kg^{-1}	1.46	1.53
K (CEC)	cmol kg^{-1}	0.36	0.99
Na (CEC)	cmol kg^{-1}	˂0.04	˂0.04
Al (CEC)	cmol kg^{-1}	˂0.06	˂0.06
Fe (CEC)	cmol kg^{-1}	˂0.01	˂0.01
Mn (CEC)	cmol kg^{-1}	˂0.01	˂0.01
H (CEC)	cmol kg^{-1}	0.002	0.002
Fe (EDTA)	mg kg^{-1}	40	67
Mn (EDTA)	mg kg^{-1}	107	128
Cu (EDTA)	mg kg^{-1}	7.2	7.1
Zn (EDTA)	mg kg^{-1}	2.3	7.5

Adapted from Prommer et al. (2014)

soils also showed an enhancement in the mineral content such as K, Ca, Mg, NH_4-N and NO_3-N, etc. as compared to the control (Agegnehu et al. 2015; Jien and Wang 2013). Biochar significantly increased soil C by 7 % (Mukherjee et al. 2014). In addition, the incubation about 3–4 months after biochar application indicates an increase in the nutrient status of highly weathered soils (Agegnehu et al. 2015; Jien and Wang 2013). The information concerning impact of biochar application on chemical properties of soil is still in an incipient stage; therefore, further research and investigation are required in the area.

Application of biochar in agriculture fields improves soil physical quality for crop production such as electrical conductivity (EC) and WHC. Humus level also increases in the amended soil due to the activity of soil microflora. Therefore, improved soil properties increase the level of nutrients available for the crops. Jien and Wang (2013) reported that addition of biochar in soil decreases the bulk density as compared to control; Mukherjee et al. (2014) also reported that biochar application increased subnanopore surface area of soil by 15 % and reduced soil bulk density by 13 % compared to control. It is reported that biochar-amended soil has an 11 % higher porosity than the unamended soil (Gul et al. 2015). Therefore, biochar plays an effective role to supporting environmental changes with soil microflora and reduction of methane gas emission in soil. The effect of biochar on soil pH and cation exchange capacity may be minimal. Prommer et al. (2014) applied three amendments in silty-loam soil 0.5 % (w/w) in triplicated plots of paddy field: Biochar (oak woodchip), Humic acid (HA) and water treatment residual (WTR) and reported that all amendments significantly augmented soil pH, nevertheless the impact of biochar was the immense. The above results are based on short-term investigation study about the impact of biochar application on soils properties. However, long-term studies with respect to use of biochar on soil physico-chemical properties are yet to be investigated.

7.6.3 Microbial Biomass of Soil

Soil microbial biomass is the key indicator of soil productivity and microbial diversity. The microbial biomass is not only responsible for carrying the nutrient cycles in paddy ecosystems, including carbon (C), nitrogen (N) and phosphorus (P) but also plays a significant role in soil nutrient transformations and acting as a labile nutrient pool offered to plants (Liu et al. 2010). Microbial biomass is responsive to biochar application to the soil of agriculture fields. As the stability period of biochar in soil is assumed to be many years, the changes in microbial biomass size and properties may continue for a long period. Jien and Wang (2013) found some changes in soil microbial activity and microbial biomass after biochar treatment. The highest contents of MBC were found at 21 days for each treated plots, which were 3200 mg kg^{-1} for 5 % biochar-amended soil, 1145 mg kg^{-1} for 2.5 %

biochar-amended soil and 1759 mg kg^{-1} for the control, respectively. The pH in the 5 % biochar-amended soil is more suitable for the growth of microbes, particularly for fungal hyphae. Wuddivira et al. (2009) demonstrated that because of higher porosity the biochar-treated soil creates suitable condition for the microbial growth and activity. Biochar has a high concentration of macropores that extends from the surface to the interior and minerals and small organic particles might accumulate in these pores. The increase in microbial biomass as a result of biochar amendment can help detect the presence of a given microbial genera or species via DNA/RNA-based techniques, due to increase in their population size and density in the soil matrix (Gul et al. 2015). This indicated that application of biochar in agriculture could maintain microbial activity in the soils for a longer period. The application of biochar may be considered as a soil conditioner as well as enhancing the microbial activity in benefits of agriculture and environment.

7.6.4 Soil Nitrification

Biochar amendment causes primary changes in soil nutrient cycles, commonly resulting in marked enhancement in crop yields, mostly in saline and unproductive soils having poor soil organic matter contents (Prommer et al. 2014). Prommer et al. (2014) reported that biochar application increased total soil organic carbon but decreased the extractable organic C pool and soil nitrate. Although gross organic N transformation rates were reduced by 50–80 %, the gross N mineralisation process remains unaffected. Biochar application increases the ammonia oxidisers population in soil and consequently more than twofold higher in nitrification rates noted (Ball et al. 2010). Prommer et al. (2014) suggested that addition of any inorganic fertiliser with the combination of biochar may compensate the reduction in organic N mineralisation and as a consequent accelerate the belowground build-up of organic N.

Biochar applications have significant effects on microbial-mediated N transformations (Ball et al. 2010) and ammonia- and methane-oxidising bacterial community composition in paddy soil (Ball et al. 2010). Changes in pH that can start similar responses in soil were not able to explain the observed changes in nitrification. Prommer et al. (2014) after applying biochar, ammonium level increased 0.001 mg kg^{-1} in the conventionally managed soils (about 88 mg kg^{-1} dry soils) compared with the organic soils (about 9 mg kg^{-1} dry soil). After increasing biochar application rate ammonium contents became 66, 30 and 15 mg kg^{-1}, respectively, but does not show significant reductions from the small initial ammonium contents in the organically managed soil. Initial nitrate contents of 5 mg kg^{-1} increased over the 60 days. Study showed that single or combined application of biochar with any inorganic fertiliser may increase soil organic N in turn enhancing soil carbon sequestration and thereby could play a significant role in future soil and environmental management planning (Prommer et al. 2014).

7.6.5 *Soil Mycorrhizal Fungi*

Biochar and mycorrhizal applications have been contributing to the sustainable crop production, ecosystem restoration, and soil carbon sequestration and mitigation of methane emission (Warnock et al. 2007). Mycorrhizal fungi are ubiquitous key indicator in nearly all terrestrial vegetation and crop systems, showing a very high degree of specificity and mutualism, enhancing plant growth. Biochar incorporation in soil has a positive impact on mycorrhizal fungi that may influence the nutrient absorption by plant roots (Ishii and Kadoya 1994; Warnock et al. 2007). Biochar can also increase endomycorrhizal plant associations that could enhance P availability in soil (Atkinson et al. 2010). In biochar-amended soil, the favourable soil conditions enhance the ability of MF to resist against plant-fungal pathogen infection through enhanced root colonisation (Atkinson et al. 2010). A number of investigations examined that biochar may influence the mycorrhizal population in terrestrial and paddy ecosystem (Warnock et al. 2007; Ishii and Kadoya 1994) but biochar application in soil and its effect on the diversity of mycorrhizal fungi is still not clear and hence there is need of further detailed study.

7.7 Impact of Biochar on Methanogens and Methanogenesis in Paddy Ecosystem

Biochar amendment affects the methanogenic archaeal community compositions in paddy soils (Dong et al. 2013). No statistically significant differences in methanogenic activities are noted in the rhizosphere of biochar amended and control soil during the rice growing seasons (Dong et al. 2013). But in a field experiment biochar addition at the rate of 9 t ha^{-1} significantly decreased CH_4 emission without affecting the CO_2 and N_2O emissions (Karhu et al. 2011). But in a laboratory incubation experiment the CH_4 emission from paddy soil was completely inhibited compared with the non-amendment control soil (Liu et al. 2011; Bosse and Frenzel 1997). Feng et al. (2012) also reported that amendment of wheat straw biochar significantly reduced CH_4 emission from paddy ecosystem. Liu et al. (2011) found that CH_4 emission from a rice paddy field was significantly increased (compared with the non-amendment control soil) in the first year after biochar amendment but was not as prominent as in the next year. It has been observed that soil CH_4 emission in response to the biochar amendment may vary with biochar types and properties. Most of the studies supported that decreasing methanogenic activity in paddy soil amended with biochar could be due to the increase in porosity of soil in presence of biochar that may inhibit the growth and multiplication of anaerobic methanogens. Although by using rice straw instead of biochar in soil, the rate of methanogenesis can be enhanced because readily degradable carbon in rice straw offered more substrates to methanogenesis to generate CH_4 than that in rice straw biochar. In contrast, there was no significant increase in CH_4 emissions associated with biochar

amendment due to their resistance to decomposition (Liu et al. 2011). However, there is no considerable information about biochar application in paddy fields related to methanogenic activity; methanogens diversity decreases with biochar amendments hence there is need of detailed study on this aspect.

7.7.1 Methane-Producing Bacteria (Methanogens)

Methanogenic archaea (methanogens) are strictly anaerobic microbes that play a vital role in anoxic environments of flooded paddy soil in the generation of CH_4 and CO_2 (Conrad 1999). Methanogens use acetate (contributes about 80% to CH_4 production) as a carbon substrate, but another substrate like H_2/CO_2 and formats also accelerate 10–30 % CH_4 production. According to Methanobacteriales, Methanococcales and Methanomicrobiales orders of methanogens have the ability to fix molecular nitrogen as they have the *nif* genes (Dannenberg and Conrad 1999). Methane is produced in the anaerobic layers of paddy soil mediated by bacterial decomposition of organic and plant residues (Dubey 2011). The characteristics of methanogens that carried anaerobic degradation of organic matter are described in Table 7.3. Methanogenesis from all substrates requires some unique coenzymes, some of which are exclusively found in methanogens (Ludmila et al. 1998; Yao and Conrad 2001). At least nine methanogen-specific enzymes are involved in the pathway of methane formation from H_2 and CO_2 (Shima 1998). In paddy soil, acetate and H_2 are the two main intermediate precursors for CH_4 formation (Yao and Conrad 1999).

Table 7.3 Some important characteristics of methanogens

Characteristics	Methanogens
Cell form	Rods, cocci, spirilla, filamentous, sarcina
Gram stain reaction	Gram +/–
Classification	*Archaebacteria*
Cell wall	pseudomurein, protein, heteropolysaccharide
Metabolism	Anaerobic
Energy and carbon source	H_2+CO_2; H_2+methanol; formate; methylamines; methanol, acetate
Catabolic products	CH_4 or CH_4+CO_2
TCA cycle	Incomplete
Carbon assimilation pathways	TCA cycle, gluconeogenesis
GC content %	26–60
Typical species	*Methanobacterium bryantii*
	Methanobrevibacter smithii
	Methanomicrobium mobile
	Methanogenium cariaci

Adapted from Dubey et al. (2005)

7.7.2 *Methanogenesis*

Biochar affects methanogenesis because numbers of methanogens reduced in anaerobic environments where sulphate and nitrate present in low concentration complete mineralisation of organic matter take place through methanogenic fermentation, which produces CH_4 and CO_2 according to reaction: $C_6H_{12}O_6 \rightarrow 3\ CO_2 + 3\ CH_4$ (Fig. 7.3). Four types of microorganism play important roles in this transformation and convert complex molecules into their simpler forms (Mer and Roger 2001). The transformation takes place by the following steps.

- Hydrolysis of biological polymers into monomers (glucides, fatty acids, amino acids) by an hydrolytic microflora that can be either aerobic, or facultatively, or strictly anaerobic;
- Acidogenesis from monomeric compounds and intermediary compounds formed during fermentation (production of volatile fatty acids, organic acids, alcohols, H_2 and CO_2) by a fermentative microflora that can be either facultatively or strictly anaerobic.
- Acetogenesis from the previous metabolites by a syntrophic or homoacetogenic microflora; and
- Methanogenesis from the simple compounds that can be used by methanogens (in particular, $H_2 + CO_2$ and acetate) which constitutes the last step of the methanogenic fermentation.

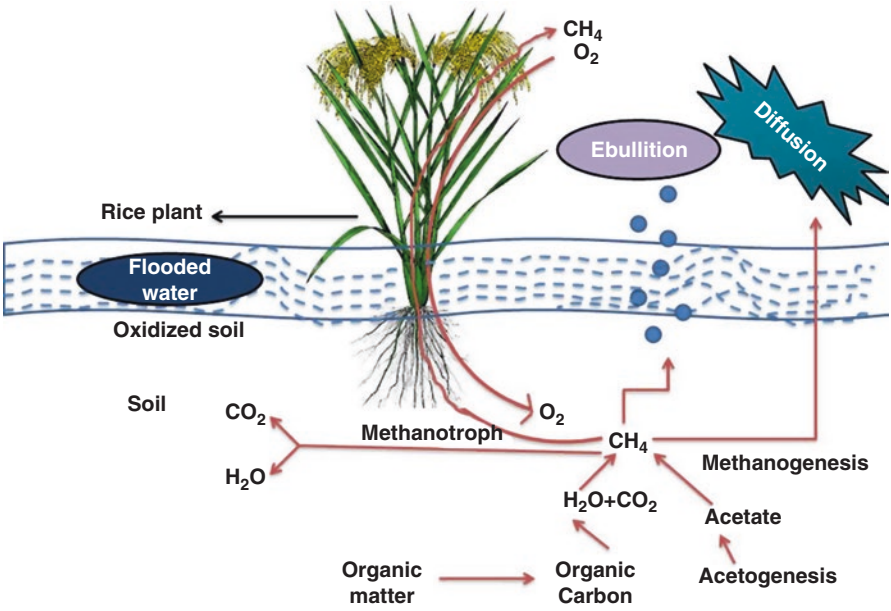


Fig. 7.3 Production, consumption and transfer of CH_4 to the atmosphere in paddy fields. Modified from Mer and Roger (2001)

Methanogens have a limited trophic spectra comprised of a small number of simple substrates: H_2+CO_2, acetate, formate, methylated compounds (methanol, methylamines, dimethyl sulphur) and primary and secondary alcohols.

7.8 Impact of Biochar on Methanotrophs and Methane Oxidation

Currently, biochar is used as an environmental and agriculturally supportive agent and hence many parts of world are applying it as a strong soil conditioner for the enrichment of soil nutrient status. The most important aspect related to biochar application in paddy field is the mitigation of methane emission and stimulation of the methane oxidation rate. Reddy et al. (2014) reported that variation in oxidation rates and kinetics of methane in soils depth was variable, therefore samples were taken from different depth of soils and examine that higher oxidation rate was found in upper layer of soil amended with biochar than lower depth of soil. Higher numbers of methanotrophs communities exist in upper layer of soil after amendment of biochar (Feng et al. 2012). Methanotrophs, aerobic bacteria, are present in the upper layer of soil (Reddy et al. 2014; Feng et al. 2012). According to Zhang et al. (2012), biochar plays significant role in the reduction of greenhouse gases mostly methane emissions in paddy soil. The different rates of greenhouse gas emissions in biochar-amended soil are presented in Fig. 7.4.

Biochar plays a significant role in methane mitigation with promoting the methanotrophs population and reducing diversity of methanogens. Paddy is one of the largest anthropogenic sources of CH_4 (6–29 % total methane emission) (Neue 1993). Mukherjee and Lal (2013) reported that biochar amendment in soil

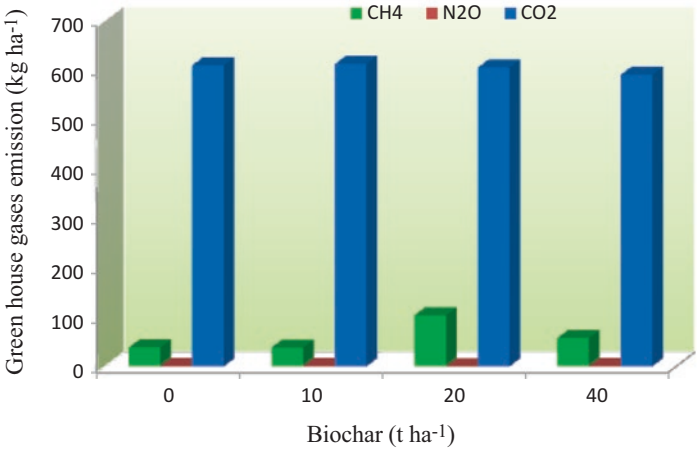


Fig. 7.4 Greenhouse gas (kg ha^{-1}) emissions from paddy field after biochar amendment. Modified from Zhang et al. (2012)

increases the aeration and porosity therefore, production of CH_4 decreases and oxidation of CH_4 increases. Furthermore, the aerobic, well-drained soils due to biochar applications can be a sink for CH_4 due to the CH_4 diffusion and subsequent oxidation by methanotrophs. Hence two mechanisms are involved here: (1) decrease the CH_4 production, and (2) increase the CH_4 oxidation by methanotrophs may be operational in the biochar-amended soil (Mukherjee and Lal 2013; Zwieten et al. 2009. According to Jien and Wang (2013) increase in soil microbes, nitrogen and phosphorus was observed after 63 and 105 days of biochar application. The highest contents of microbial carbon were found at 21 days for each treated soil, which were 3200 mg kg^{-1} for 5 % biochar-amended soil (Jien and Wang 2013). This shows that amendment of biochar in soil supports the microbial growth, mostly methanotrophs which play significant role in CH_4 uptake. Therefore, an effective process to decrease CH_4 emission in paddy soil may be application of biochar (Lehmann 2007). Previous work has shown that CH_4-oxidising bacteria are readily enriched within landfill cover soil by exposure to the CH_4 generated from the waste (Reddy et al. 2014).

7.8.1 Methanotrophs or Methane-Oxidising Bacteria

Methanotrophs are Gram-negative bacteria that utilise CH_4 as their sole source of carbon and energy play a crucial role in reducing global CH_4 load due its CH_4 consumption characteristics. Studies on CH_4 sink measurement from various agro and natural ecosystems showed that the soils of these ecosystems exhibited a significant variation in CH_4 sink activity due to methanotrophic bacteria. Paddy soil methanotrophic communities exhibit the highest CH_4 sink activity on a global scale (Tiwari et al. 2015). Based on physiology, phylogeny, biochemistry, resting stage, intracellular membrane, genetic characters, ultrastructure and phospholipid ester-linked fatty acid (PLFA) analyses of 14 culturable genera (Han et al. 2009) of aerobic proteobacterial methanotrophs are classified as type I belongs to Gammaproteobacteria group and contain genera *Methylobacter, Methylomonas, Methylosphaera, Methylomicrobium, Methylothermus, Methylosarcina, Methylohalobius,* and *Methylosoma* while type II belongs to Alphaproteobacteria group of CH_4-oxidising bacteria and include genera *Methylocystis, Methylosinus, Methylocapsa, Methylocella*. Type I group of methanotrophs is further subdivided into types Ia and Ib (Bodrossy et al. 2003; Krause et al. 2010). Type I subgroup contains several culturable methanotrophs, for example *Methylomonas*, *Methylosarcina*, *Methylobacter*, etc. However, *Methylocaldum and Methylococcus* come under the subgroup Type Ib or rare type X (Hanson and Hanson 1996; Graef et al. 2011; Giri et al. 2014; Tiwari et al. 2015). Type I methanotrophs also referred as 'high capacity–low affinity' methanotrophs are adapted for high CH_4 concentrations and assimilate it through RuMP pathway whereas Type II is generally termed as 'low capacity–high affinity' methanotrophs capable of using trace quantity of CH_4 from the environment and follow the serine pathway for CH_4 oxidation (Hanson and Hanson 1996; Tiwari

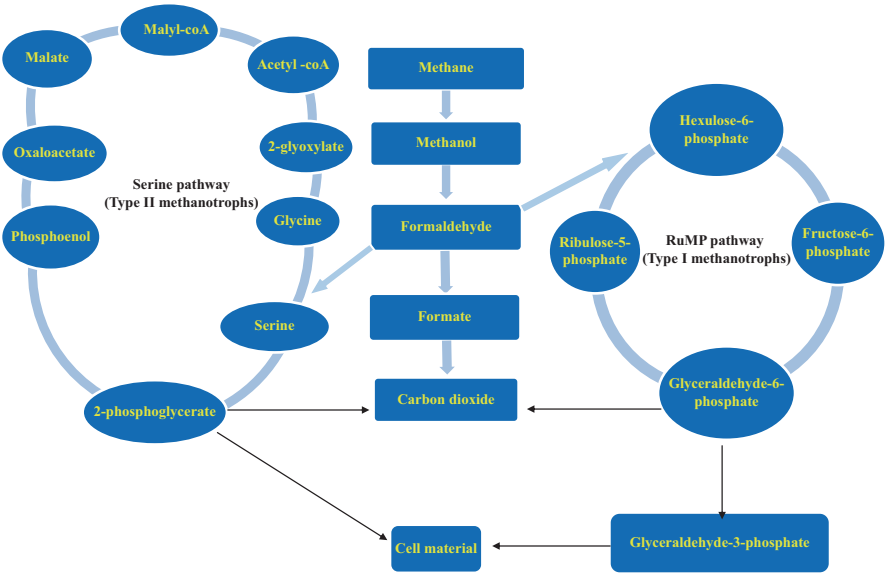


Fig. 7.5 Oxidation pathway of Type I and Type II methanotrophs

et al. 2015). *Verrucomicrobia*, a new group of CH_4 oxidiser discovered in recent past involved in methane oxidation (Siljanen et al. 2011; Luke et al. 2011; Graef et al. 2011; Tiwari et al. 2015) The methane oxidation pathways by Type I and Type II methanotrophs is presented in Fig. 7.5.

Singh (2010) reported that during last 10 years the extensive study has been done related to population dynamics and diversity of methanotrophic genera bacteria. Currently, 18 genera of cultivated aerobic methanotrophs (Gammaproteobacteria) and five genera of Alphaproteobacteria are represented by approximately 60 different species of the bacteria (Singh 2010). Rising temperature around the earth's surface is directly associated with the increasing atmospheric level of water vapour, CO_2, CH_4, N_2O, SF_6, etc. due to anthropogenic activities (IPCC 2007; EPA 2010; Krause et al. 2010; Li and Wang 2013). Though the atmospheric concentration of CH_4 is extremely less than CO_2 (IPCC 2007), CH_4 is more efficient to trap radiation than CO_2 (Solomon et al. 2007; Siljanen et al. 2011; Pandey et al. 2014). It is assumed that methane, 27 times potent GHG than CO_2 (Houghton et al. 1995; Phillips et al. 2001; Singh and Gupta 2016), accounting about 15–20 % of the global warming effect (Phillips et al. 2001; Wuebbles and Hayhoe 2002; Jang et al. 2006; IPCC 2007; Dalal and Allen 2008; Tiwari et al. 2015). Being highly reactive in nature, CH_4 affects the chemistry and oxidation capacity of the environment by influencing the level of CO, OH^-, tropospheric ozone, etc. (Cicerone and Oremland 1988). Global atmospheric concentration of CH_4 has almost tripled since pre-industrial times (Krause et al. 2010) increasing rate up to 0.5–1 % $year^{-1}$

(IPCC 2001, 2007; Tamai et al. 2007; Tiwari et al. 2015). The annual release of CH_4 into the atmosphere was 180 Tg year^{-1} (Khalil and Rasmussen 1994; Mer and Roger 2001; Hill et al. 2016).

In global perspective, most of the atmospheric CH_4 is eliminated from the environment through chemical reactions with hydroxyl radicals (OH^-) in the troposphere ($CH_4 + OH^- \rightarrow CH_3^- + H_2O$), and in stratosphere CH_4 reacts with the chlorine originated from CFCs (Chlorofluorocarbons) ($CH_4 + Cl^- \rightarrow HCl + CH_3^-$.) which involve around 90 % of the total Global CH_4 sinks (Schlesinger 1997; IPCC 2001; Hutsch 2001, Mer and Roger 2001; Tiwari et al. 2015). Mer and Roger (2001) state that if equilibrium between by methanogens CH_4 emission and methanotrophs CH_4 oxidation is positive, the environment may be a CH_4 source and if the equilibrium is negative the environment may be a CH_4 sink. Aerobic soils are the important biological sink for CH_4 due to the presence of unique methanotrophic bacteria (Singh 2010; Tiwari et al. 2015). Methanotrophs utilise CH_4 as their carbon and electron source from the surrounding environment. The estimated amount of CH_4 consumed by methanotrophic bacteria is between 10 and 40 Tg year^{-1} and comprises approximately 6–10 % of the total CH_4 oxidation of the atmosphere (IPCC 2001; Tiwari et al. 2015). Up to 95 % of the CH_4 emitted anoxically may be consumed before destined into the atmosphere (Frenzel et al. 1990; Graef et al. 2011). Therefore, even minute alteration in consumption capacity may have a global significance if key regions such as the Arctic and Antarctica are concerned (Graef et al. 2011). It is assumed that 10–30 % of the CH_4 emitted by methanogenic bacteria in submerged conditions of paddy fields is oxidised by methanotrophs linked with the roots of rice crop (King 1997; Schlesinger 1997; IPCC 2001; Mohanty et al. 2007; Tiwari et al. 2015).

7.9 Conclusions and Future Research Directions

Results indicate that biochar and/or compost in a range of combinations added as soil amendments with supplementary fertiliser can improve soil health and boost productivity of paddy crops with the additional environmental benefits of global warming and climate change mitigation. This approach can therefore contribute positively to agricultural and environmental sustainability. Biochar and biochar-compost applications positively impact soil fertility, for example, through their effect on soil physico-chemical properties and plant available nutrients.

Significant increases in various crop yields and plant available soil nutrients were observed due to biochar and compost addition in comparison to the fertiliser only treatment, indicating that application of organic amendments does provide agronomic benefits. The response of paddy crop to biochar and organic amendments could be due to their effects on plant available nutrients, biological N fixation, soil water and nutrient retention, although other mechanisms cannot be discounted. Study indicates that fresh biochar mitigates CH_4 emissions immediately after its addition to soil. It has been reported that biochar application to increase CH_4 uptake, probably due to better soil aeration and optimum moisture availability.

Application of biochar can significantly improve soil physical quality in terms of bulk density, aeration, porosity and WHC. Biochar has a potentially positive role to play in limiting GHGs emissions but a greater understanding of the mechanisms involved is required. The study showed that biochar addition may reduce the climate change impact of agriculture in both perennial bioenergy crop soils and arable soils. However, further research is required to confirm these results in a variety of agriculture soils using a variety of biochar types. Longer term experiments need to be conducted in order to monitor the effect of biochar on soil CH_4 emissions/consumptions following rainfall or N fertilisation events, taking measurements from the day of biochar application onwards. Future studies should investigate whether biochar applications can affect the N use efficiency of paddy agriculture and population dynamics of methanogens/methanotrophs. Additionally, future studies should analyse all of the N-based fertiliser and biochar addition to soil under a range of environmental regimes such as different soil types, N application rates and timings and repeated biochar applications. Future research should make certain that the biochar production and methods of amendments used are sustainable in a social, environmental and economic context.

References

Abdullah H, Wu HW (2009) Biochar as a fuel: properties and grindability of biochars produced from the pyrolysis of Mallee wood under slow-heating conditions. Eng Fuels 23:4174–4181

Agegnehu G, Bass AM, Nelson PN, Muirhead B, Wright G, Michael I (2015) Bird biochar and biochar-compost as soil amendments: effects on peanut yield, soil properties and greenhouse gas emissions in tropical North Queensland, Australia. Agric Ecosyst Environ 213:72–85

Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 337:1–18

Bailey VL, Fansler SJ, Smith JL, Bolton JH (2010) Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biol Biochem 43:296–301

Ball PN, MacKenzie MD, DeLuca TH, Holben WE (2010) Wildfire and charcoal enhance nitrification and ammonium-oxidizing bacteria abundance in dry montane forest soils. J Environ Qual 39:1243–1253

Bodrossy L, Stralis-Pavese N, Murrell JC, Radajewski S, Weilharter A, Sessitsch A (2003) Development and validation of a diagnostic microbial microarray for methanotrophs. Environ Microbiol 5:566–582

Bosse U, Frenzel P (1997) Activity and distribution of methane-oxidizing bacteria in flooded rice soil microcosms and in rice plants (Oryza sativa). Appl Environ Microbiol 63:1199–1207

Bruun EW, Ambus P, Egsgaard H, Hauggaard-Nielsen H (2012) Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biol Biochem 46:73–79

Carriera M, Hardieb AG, Urasa U, Gorgensa J, Knoetze JH (2012) Production of char from vacuum pyrolysis of South-African sugar cane bagasse and its characterization as activated carbon and biochar. J Anal Appl Pyrolysis 96:24–32

Cicerone RJ, Oremland RS (1988) Biogeochemical aspects of atmospheric methane. Glob Biochem Cycles 2:299–327

Conrad R (1999) Soil microorganisms oxidizing atmospheric trace gasses (CH_4, CO, H2, NO). Ind J Microbiol 39:193–203

Dalal RC, Allen DE (2008) Greenhouse gas fluxes from natural ecosystems. Aus J Bot 56:369–407
Dannenberg S, Conrad R (1999) Effect of rice plant on methane production and rhizospheric metabolism in paddy soil. Biogeochemistry 45:53–71
Demirbas A (2001) Biomass resource facilities and biomass conversion processing for fuels and chemicals. Energ Conver Manag 42:1357–1378
Demisie W, Zhang M (2015) Effect of biochar application on microbial biomass and enzymatic activities in red soil. African J Agric Res 10:755–766
Dong D, Yang M, Wang C, Wang H, Li Y, Luo J, Wu W (2013) Responses of methane emissions and rice yield to applications of biochar and straw in a paddy field. J Soil Sediment 13:1450–1460
Dubey SK (2011) Methane emission and rice agriculture. Curr Sci 81:345–346
EPA (2010) Methane and nitrous oxide emission from natural sources. U.S. Environmental Protection Agency, Washington
Eykelbosh AJ, Johnson MS, Queiroz ESD, Dalmagro HJ, Couto EG (2014) Biochar from sugarcane filtercake reduces soil CO_2 emissions relative to raw residue and improves water retention and nutrient availability in a highly-weathered tropical soil. PLoS One 9:98523
Feng Y, Xu Y, Yu Y, Xie Z, Lin X (2012) Mechanisms of biochar decreasing methane emission from Chinese paddy soils. Soil Biol Biochem 46:80–88
Frenzel P, Therbrath B, Conrad R (1990) Oxidation of methane in the toxic surface layer of deep lake sediment (Lake Constance). FEMS Microbiol Ecol 73:149–158
Gaunt JL, Johannes L (2008) Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. Environ Sci Technol 42:11–4152
Ghoneim AM, Ebid AI (2013) Impact of rice-straw biochar on some selected soil properties and rice (*Oryza sativa L.*) grain yield. Int J Agron Agric Res 3:14–22
Giri DD, Kumar A, Sahu PK, Mishra PK, Pandey KD (2014) Temperature dependent decline in soil methane oxidizing bacterial population in tropical dry deciduous forest ecosystems. Int J Sci Technol Res 3:2277–8616
Graef C, Hestnes AG, Svenning MM, Frenzel P (2011) The active methanotrophic community in a wetland from the high arctic. Environ Microbiol Rep 3:466–472
Gul S, Whalen JK, Thomas BW, Sachdeva V, Deng H (2015) Physico-chemical properties and microbial responses in biochar-amended soils mechanisms and future directions. Agric Ecosyst Environ 206:46–59
Han B, Chen Y, Abell Y, Jiang H, Bodrossy L, Zhao J, Murrel JC, Xing X (2009) Diversity and activity of methanotrophs in alkaline soil from a Chinese coalmine. FEMS Microbiol Ecol 70:196–207
Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471
Hmid A, Mondelli D, Fiore S, Fanizzi FP, Ziad Al C, Dumontet S (2014) Production and characterization of biochar from three-phase olive mill waste through slow pyrolysis. Biomass Bioener 71:330–339
Houghton JT, Filho LGM, Bruce J, Lee H, Callander BA, Haites E, Harris N, Maskell K (1995) Radiative forcing of climate change. In: van der Linden PJ, Hanson CE (eds) Climate change. Cambridge University Press, Cambridge
Hutsch BW (2001) Methane oxidation, nitrification, and counts of methanotrophic bacteria in soils from a long-term fertilization experiment ('Ewiger Roggenbau' at Halle). J Plant Nutr 164:21–28
Intergovernmental Panel on Climate Change (2001) The science of climate change In: Climate change 2001. Cambridge University Press, Cambridge
Intergovernmental Panel on Climate Change (2007) The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. In: Climate change 2007: climate change and environmental sustainability. Cambridge University Press, New York, pp 433–497
Ishii T, Kadoya K (1994) Effects of charcoal as a soil conditioner on citrus growth and vesicular-arbuscular mycorrhizal development. J Jpn Soc Hortic Sci 63:529–535

Jang I, Lee S, Hong JH, Kang H (2006) Methane oxidation rates in forest soils and their controlling variables: a review and a case study in Korea. Ecol Res 21:849–854
Jien SH, Wang CS (2013) Effects of biochar on soil properties and erosion potential in a highly weathered soil. Catena 110:225–233
Jindo K, Suto K, Matsumoto K, García C, Sonoki T, Sanchez-Monedero MA (2012) Chemical and biochemical characterisation of biochar-blended composts prepared from poultry manure. Bioresour Technol 110:396–404
Johannes L (2007) Bio-energy in the black. Front Ecol Environ:5–7
Jouiada M, Al-Nofeli N, Khalifa N, Benyettouc F, Yousef LF (2015) Characteristics of slow pyrolysis biochars produced from rhodes grass and fronds of edible date palm. J Anal Appl Pyrolysis 111:183–190
Karhu K, Mattila T, Bergstrom I, Regina K (2011) Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity-results from a short-term pilot field study. Agric Ecosyst Environ 140:309–313
Keppler F, Hamilton JTG, Brab M, Rockmann T (2006) Methane emissions from terrestrial plants under aerobic conditions. Nature 439:187–191
Khalil MAK, Rasmussen RA (1994) Global emissions of methane during the last several centuries. Chemosphere 29:833–842
Khan ST, Mubeen U (2012) Wheat straw: a pragmatic overview. Curr Res J Biol Sci 4:673–675
Kim SS, Agblevor FA, Lim J (2009) Fast pyrolysis of chicken litter and turkey litter in a fluidized bed reactor. J Ind Eng Chem 15:247–252
King GM (1997) Responses of atmospheric methane consumption by soils to global climate change. Glob Chang Biol 3:351–362
Krause S, Ke CL, Frenzel P (2010) Succession of methanotrophs in oxygen–methane countergradients of flooded rice paddies. Int Soc Microb Ecol J4:1603–1607
Lai W-Y, Lai C-M, Ke G-R, Chung R-S, Chen C-T, Cheng C-H, Pai C-W, Chen S-Y, Chen C-C (2013) The effect of woodchip biochar application on crop yield, carbon sequestration and greenhouse gas emission from soils planted with rice or leaf beet. J Taiwan Inst Chem Eng 44:1039–1044
Laird DA (2008) The Charcoal vision: a win–win–win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. J Agron
Lehmann J (2007) A handful of carbon. Nature 447:143–144
Lehmann J, Joseph S (2009) Biochar for environmental management an introduction. ES-BEM-16:17–23
Li YL, Wang XX (2013) Root-induced changes in radial oxygen loss, rhizosphere oxygen profile, and nitrification of two rice cultivars in Chinese red soil regions. Plant and Soil 36:115–126
Liu S, Li Y, Wu J, Huang D, Su Y, Wei W (2010) Spatial variability of soil microbial biomass carbon, nitrogen and phosphorus in a hilly red soil landscape in subtropical China. Soil Sci Plant Nutr 56:693–704
Liu YX, Yang M, Wu YM, Wang HL, Chen YX, Wu WX (2011) Reducing CH_4 and CO_2 emissions from waterlogged paddy soil with biochar. J Soil Sediment 11:930–939
Ludmila C, Julia A, Rudoyk RS, Toms M, Lidstrom E (1998) G transfer enzymes and coenzymes were linking methylotrophic bacteria and methanogenic archaea. J Sci 281:99–101
Luke C, Bodrossy L, Lupotto E, Frenzel P (2011) Methanotrophic bacteria associated to rice roots: the cultivar effect assessed by T-RFLP and microarray analysis. Environ Microbiol Rep 3:518–525
Mahinpey N, Murugan P, Mani T, Raina R (2009) Analysis of bio-oil, biogas, and biochar from pressurized pyrolysis of wheat straw using a tubular reactor. Energy Fuel 23:2736–2742
Masulili A, Utomo WH (2010) Rice husk biochar for rice based cropping system in acid soil 1. The characteristics of rice husk biochar and Its Influence on the properties of acid sulfate soils and rice growth in West Kalimantan, Indonesia. J Agric Sci 2:1
McLaughlin S, Walsh M (1998) Evaluating environmental consequences of producing herbaceous crops for bioenergy. Biomass Bioener 14:317–324

Mer JL, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. Eur J Soil Biol 37:25–50
Meyer S, Glaser B, Quicker P (2011) Technical, economical and climate related aspects of biochar production technologies: a literature review. Environ Sci Technol 45:9473–9483
Milla OV, Rivera EB, Huang WJ, Chien CC, Wang YM (2013) Agronomic properties and characterization of rice husk and wood biochars and their effect on the growth of water spinach in a field test. J Soil Sci Plant Nutr 13:251–266
Mohammed IY, Abakr YA, Kazi FK, Yusuf S, Alshareef I, Chi SA (2015) Pyrolysis of Napier grass in a fixed bed reactor: effect of operating conditions on product fields and characteristic. Bio Res 10:6457–6478
Mohanty SR, Bodelier PLE, Conrad R (2007) Effect of temperature on composition of the methanotrophic community in rice field and forest soil. FEMS Microbiol Ecol 62:24–31
Mukherjee A, Lal R (2013) Biochar impacts on soil physical properties and greenhouse gas emissions. Agronomy 3:313–339
Mukherjee A, Lal R, Zimmerman AR (2014) Effects of biochar and other amendments on the physical properties and greenhouse gas emissions of an artificially degraded soil. Sci Total Environ 487:26–36
Neue NU (1993) Methane emission from rice fields. Bioscience 43:466–474
Pan GX, Zhou P, Li ZP, Smith P, Li LQ, Qiu DS, Zhang XH, Xu XB, Shen SY, Chen XM (2009) Combined inorganic/organic fertilization enhances N efficiency and increases rice productivity through organic carbon accumulation in a rice paddy from the Tai Lake region, China. Agric Ecosyst Environ 131:274–280
Pandey VC, Singh JS, Singh DP, Singh RP (2014) Methanotrophs: promising bacteria for environmental remediation. Int J Environ Sci Technol 11:241–250
Peter W (2007) Biochar and bioenergy production for climate change mitigation. New Zealand Sci Rev 64
Phillips R, Whalen SC, Schlesinger WH (2001) Influence of atmospheric CO_2 enrichment on methane consumption in a temperate forest soil. Glob Chang Biol 7:557–563
Prommer J, Wanek W, Hofhansl F, Trojan D, Offre P, Urich T, Schleper C, Sassmann S, Kitzler B, Soja G, Hood-Nowotny RC (2014) Biochar decelerates soil organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial. PLoS One 9:86388
Reddy KR, Asce F, Yargicoglu EN, Asce SM, Yue D, Yaghoubi P (2014) Enhanced microbial methane oxidation in landfill cover soil amended with biochar. J Geotech Geoenviron Eng 11:1090–0241
Sadaka S, Sharara MA, Ashworth A, Keyser P, Allen F, Wright A (2014) Characterization of biochar from switchgrass carbonization. Energy 7:548–567
Schlesinger HW (1997) Biogeochemistry: an analysis of global change. Academic Press, New York
Shackley S, Carter S, Knowles T, Middelink E, Haefele S, Sohi S, Cross A, Haszeldine S (2012) Sustainable gasification–biochar systems? A case-study of rice-husk gasification in Cambodia, Part I: context, chemical properties, environmental and health and safety issues. Energy Policy 42:49–58
Shima SL (1998) Mechanism of biological methane formation: structure and function of methylcoenzyme M reductase. Prote Nucleic Acid Enzyme 43:1461–1467
Siljanen HMP, Saari A, Krause S, Lensu A, Abell GCJ, Bodrossy L, Bodelier PLE, Martikainen PJ (2011) Hydrology is reflected in the functioning and community composition of methanotrophs in the littoral wetland of a boreal lake. FEMS Microbiol Ecol 75:430–445
Singh JS (2010) Methanotrophs: the potential biological sink to mitigate the global methane load. Curr Sci 100:1–10
Singh JS, Gupta VK (2016) Degraded land restoration in reinstating CH_4 sink. Front Microbiol 7(923):1–5
Solomon S, Qin D, Manning M, Alley RB, Berntsen T, Bindoff NL, Chen Z, Chidthaisong A, Gregory JM, Hegerl GC, Heimann M, Hewitson B, Hoskins BJ, Joos F, Jouzel J, Kattsov V, Lohmann U, Matsuno T, Molina M, Nicholls N, Overpeck J, Raga G, Ramaswamy V, Ren J, Rusticucci M, Somerville R, Stocker TF, Whetton P, Wood RA, Wratt D (2007) Technical

summary. In: Solomon SD, Qin M, Manning Z, Chen M, Marquis KB, Averyt M, Tignor HL, Miller (eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

Songa W, Guo M (2012) Quality variations of poultry litter biochar generated at different pyrolysis temperatures. J Anal Appl Pyrolysis 94:138–145

Spokas KA, Koskinen WC, Baker JM, Reicosky DC (2009) Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere 77:574–581

Tamai N, Takenaka C, Ishizuka S (2007) Water soluble Al inhibits methane oxidation at atmospheric concentration levels in Japanese forest soil. Soil Biol Biochem 39:1730–1736

Tiwari S, Singh JS, Singh DP (2015) Methanotrophs and CH_4 sink: effect of human activity and ecological perturbations. Clim Change Environ Sustainabil 3:35–50

Warnock DD, Lehmann J, Kuyper TW, Rillig MC (2007) Mycorrhizal responses to biochar in soil concepts and mechanisms. Plant Soil 300:9–20

Wu F, Jia Z, Wang S, Chang SX, Startsev A (2013) Contrasting effects of wheat straw and its biochar on greenhouse gas emissions and enzyme activities in a chernozemic soil. Biol Fertil Soils 49:555–565

Wu M, Feng Q, Sun X, Wang H, Gielen G, Wu W (2015) Rice (*Oryza sativa L*) plantation affects the stability of biochar in paddy soil. Scientific Report 5, Article number: 10001

Wuddivira MN, Stone RJ, Ekwue EI (2009) Structure stability of humid tropical soils as influenced by manure incorporation and incubation duration. Soil Sci Soc Am J 73:1353–1360

Wuebbles DJ, Hayhoe K (2002) Atmospheric methane and global change. Earth Sci Rev 57:177–210

Yamato M, Okimori Y, Wibowo IF, Anshori S, Ogawa M (2006) Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut and soil chemical properties in south Sumatra, Indonesia. Soil Sci Plant Nutr 52:489–495

Yang Y, Ma S, Zhao Y, Jing M, Xu Y, Chen J (2015) A field experiment on enhancement of crop yield by rice straw and corn stalk-derived biochar in Northern China. Sustain 7:13713–13725

Yao H, Conrad R (1999) Thermodynamics of methane production in different rice paddy soils from China, the Philippines, and Italy. Soil Biol Biochem 31:463–473

Yao H, Conrad R (2001) Thermodynamics of propionate degradation in anoxic paddy soil from different rice-growing regions. Soil Biol Biochem 33:359–364

Yargicoglu EN, Sadasivam BY, Reddy KR, Spokas K (2015) Physical and chemical characterization of waste wood derived biochars. Waste Manag 36:256–268

Zhang XC, Liu XH (2012) Effect of biochar on pH of alkaline soils in the loess plateau: results from incubation experiments. Int J Agric Biol 14:745–750

Zhanga A, Biana R, Pana G, Cuia L, Hussaina Q, Li L, Jinwei Z, Zhenga J, Zhanga X, Xiaojun H, Yua X (2010a) Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a field study of 2 consecutive rice growing cycles. Field Crop Res 127:153–160

Zhanga A, Cuia L, Pana G, Li L, Hussaina Q, Zhanga X, Zhenga J, Crowley D (2010b) Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. Agric Ecosyst Environ 139:469–475

Zwieten V, Singh LB, Joseph S, Kimber S, Cowie A, Yin Chan K (2009) Biochar and emissions of non-CO_2 greenhouse gases from soil. In: Lehmann J, Joseph S (eds) Biochar for environmental management. Earthscan, London

Zwieten VL, Kimber S, Morris S, Chan YK, Downie A, Rust J, Joseph S, Cowie A (2010) Effect of biochar from slow pyrolysisi of papermill waste on agronomic performance and soil fertility. Plant Soil 327:235–246

Chapter 8
Role of Rhizospheric Microbes in Heavy Metal Uptake by Plants

Mihiri Seneviratne, Gamini Seneviratne, HMSP Madawala, and Meththika Vithanage

Abstract Due to industrialization, excessive use of pesticides and fertilizer and improper waste management practices cause heavy metal accumulation in both soil and water. Due to the nondegradable and persistent nature, heavy metals can be accumulated in soils for hundreds of years. They enter the bodies of plants and animals and thereby cause negative health impacts to the environment. Even though the soil heavy metal remediation is a must, it is not an easy task to achieve. Among many physical and chemical methods, phytoremediation plays an important role, due to its efficient and convenient nature. Rhizophere microbes play an important role in phytoremediation. Since, rhizosphere is the immediate vicinity of the root, the chemical and physical changes in that environment can easily effect heavy metal uptake by the plant. By siderophore production, acidification, releasing plant growth promoters, reducing the plant stress conditions and through redox changes rhizosphere enhances the phytoreomediation processes. However, plants can bioconcentrate (phytoextraction) and also bioimmobilize the toxic heavy metals through rhizospheric processes. This chapter summaries the role of rhizospheric organisms for facilitation of heavy metal uptake, the different mechanisms of enhancing the availability of heavy metals in the rhizosphere, the genetic diversity, and the microbial genera that involve in these processes.

Keywords Phytoremediation • Rhizosphere • Agricultural soils • Bioimmobilization • Plant growth promoters

M. Seneviratne
Department of Botany, Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka

G. Seneviratne
Microbial Biotechnology Unit, National Institute of Fundamental Studies, Kandy, Sri Lanka

HMSP Madawala
Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka

M. Vithanage (✉)
Chemical and Environmental Systems Modelling Research Group, National Institute of Fundamental Studies, Kandy, Sri Lanka
e-mail: meththikavithanage@gmail.com

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_8

8.1 Introduction

The term heavy metals is defined as metals with high relative atomic weight and atomic number greater than 20 (Raskin et al. 1994). Some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are required in minute quantities by organisms, and they are defined as trace elements. However, heavy metals such as Pb, Cd, Hg, and As do not have any biological role in living cells, but they cause hazardous effects so that they are labeled as toxic metals. Due to industrialization, excessive use of pesticides and fertilizer and improper waste management practices cause heavy metal accumulation in both soil and water. As they enter to the soil system, they harbor in different fractions; exchangeable ions, insoluble inorganic metal compounds such as carbonates and phosphates, soluble metal compound or free metal ions in the soil solution, metal complex of organic materials, and metals attached to silicate minerals (Marques et al. 2009). Not like many other pollutants, heavy metals do not degrade either chemically or biologically. Therefore, the exchangeable fraction easily enters into the plant and animal bodies and causes bioaccumulation. Bioaccumulation of heavy metals causes a many diseases and disorders in humans, animals, and plants.

Since soil remediation is a global concern, where a variety of physical, chemical, and biological methods are used. Physical remediation techniques include soil washing and soil vapor extraction, whereas chemical remediation involves the use of chemicals to extract pollutants from contaminated media. Soil physical and chemical remediation are quite costly and need skilled labor. In biological remediation, both plants and microbes are used in the process of pollutant removal (Khan et al. 2000). Bioremediation is a cost-beneficial and an environmental friendly method.

Phytoremediation is a branch of bioremediation that uses plants for the removal of pollutants from contaminated soils. It is effective for contaminated sites with pollutants that are distributed within the root zone of the plant (Garbisu and Alkorta 2003). The rhizosphere bacteria which inhabit the root zone of the plant play an important role in phytoremdiation process, via various mechanisms.

Rhizosphere is the immediate vicinity of the root. Since most of the physical and chemical activities which take place in the rhizosphere have a direct impact on the root system. It is well understood that plant–microbe interactions determine the efficiency of metal extraction. Metalliferous plants are able to grow on trace element-enriched soils and rocks without any symptoms of toxicity. Comparisons between sterile and nonsterile soil systems showed that heavy metal accumulators achieve their full accumulation capacity only in the presence of their indigenous rhizosphere microflora (de Souza et al. 1999). Whiting et al. (2001) showed that *Thlaspi caerulescens* plants inoculated with rhizosphere bacteria accumulated the highest amounts of Zn. Different mechanisms, EPS production, rhizosphere acidification through organic acids, siderophore production, indole-3-acetic (IAA) or 1-amino-1-cyclopropanoic acid (ACC) deaminase production, or the release of growth-limiting

Table 8.1 Heavy metal adsorption by rhizospheric bacteria

Name of the organism	Heavy metal	Reference
Mesorhizobium amorphae	Zn	Hao et al. (2014)
Paenibacillus jamilae	Pb, Cd, Co, Ni, Zn, and Cu	Pérez et al. (2008)
Pseudomonas aeruginosa	Cu, Pb, Zn	Teitzel and Parsek (2003)
Azotobacter	Cd, Cr	Joshi and Juwarkar (2009)
Rhizobium etli	Mn(II)	Pulsawat et al. (2003)
Bacillus firmus	Pb, Cu, and Zn	Salehizadeh and Shojaosadati (2003)
Enterobacter cloaceae	Cr(VI)	Iyer et al. (2004)
Ralstonia	Cd	Chompoothawat et al. (2010)
Micrococcus luteus	Cu, Pb	Puyen et al. (2012)

nutrients from the soil, are involved in improving the rate of heavy metal accumulation in plants. This chapter discusses the role of rhizospheric microorganisms in heavy metal uptake, the different mechanisms engaged, the rhizospheric microorganisms involved in plant heavy metal uptake, the research needs, and the future direction.

8.2 Role of Microbial EPS Production in Bioremediation

Among many different mechanisms, extracellular polymeric substances (EPS) play a very important role in biosorption of heavy metals, and EPS are produced by most bacteria (Table 8.1). Rhizosphere bacteria produce more EPS than non-rhizospheric isolates (Kunito et al. 2001). The exopolymer production increased in the presence of Cu, and this was more prominent for the isolates from the rhizosphere. The harmful effect of Cu on the growth rate was small for the Cu-resistant bacteria which produce a high quantity of exopolymers. This was explained as the involvement of exopolymers in the detoxification of Cu (Kunito et al. 2001). Exopolymers that are produced by bacteria are able to bind strongly with trace elements (Bitton and Freihofer 1977) and form organo-metallic complexes, which are difficult to degrade or decompose naturally (Hattori 1996). Moreover, the trace element concentrations enhance the production of exopolymers (Kidambi et al. 1995). The EPS production is reported as a potential mechanism of mercury tolerance in bacteria (Cruz 2014). EPS with different chemical compositions was tested for their ability to sorb mercury, and it was observed that the EPS containing hexosamines was the most effective in removing mercury from the solution whereas EPS consisting neutral sugars removed the least amount of mercury from the solution (Cruz 2014). Studies reported that EPS producing *Azotobacter* spp. was able to bind CrO_4^{2-} and Cd^{2+} (Joshi and Juwarkar 2009). Other than bacteria, biofilms, which are communities of microorganisms, are also able to produce EPS (Flemming et al. 2007). It has been reported that bacterial EPS production is involved in many heavy metal adsorption (Lau et al. 2005). The adsorption of heavy metals is higher in bacterial biofilms

compared to their planktonic counterparts. A number of studies have shown the ability of biofilms in heavy metal adsorption (Jang et al. 2001; Ueshima et al. 2008; Wilson et al. 2001). EPS production is enhanced under low N, P, and S contains and also in the presence of high content of C (Czaczyk and Myszka 2007).

EPS comprise a mixture of polysaccharides, mucopolysaccharides, humic substances, and proteins, which depends on the strain and its culture conditions (Ahemad and Kibret 2013). The proportion of EPS in biofilms can comprise between approximately 50 and 90 % of the total organic matter (Donlan 2002). The polysaccharides in Gram-negative bacteria are neutral or polyanionic. Uronic acids or ketal-linked pyruvates enhance their anionic properties which enhance the adsorption ability of divalent cations to the biofilm (Vu et al. 2009). In some Gram-positive bacteria, the chemical composition of their EPS could be slightly different due to their cationic nature (Sutherland 2001). It was revealed that the EPS produced by Ni-resistant *Cupriavidus pauculus* bacteria isolated from serpentine soil was a homopolymer of rhamnose containing uronic acid, protein, and nucleic acid (Pal and Paul 2013).

Quorum sensing (QS) is one of the regulatory pathways for EPS production (Masák et al. 2014). QS in EPS production is a very complex process. In *Pseudomonas aeruginosa*, QS is essential for adhesion EPS production and biofilm formation. They have two QS systems, namely LasI/LasR and RhlI/RhlR. Mutant *P. aeruginosa* cells, which do not produce any QS signals were found to be more densely populated with a very thin EPS matrix compared to the wild type (Gupta and Schuster 2012). *Rhizobium meliloti* is a soil bacterium, which fixes nitrogen in symbiotic association with the leguminous plant *Medicago sativa* (Alfalfa). It produces succinoglycan as its major exopolysaccharide which is a polymer of repeating octasaccharide subunits (Leigh and Walker 1994). Similar to other EPSs succinoglycan is also originated from cytoplasmic sugars.

exoR and exoS are involved in the regulation of EPS I synthesis in the free-living state of *Rhizobium meliloti* (Reuber et al. 1991). In addition, they have discovered that *R. meliloti* has a latent capacity to synthesize a second exopolysaccharide (EPS II) that can substitute for the role(s) of EPS I in nodulation of alfalfa (Glazebrook et al. 1990). Products generated by exoR and exoS play a negative roles in EPS synthesis.

Pseudomonas sp. is a model organism which has been used to study the EPS production (Wei and Ma 2013). It has been reported that three exopolysaccharides (Psl, Pel, and alginate) are produced by *P. aeruginosa.* The Psl cluster consists of 15 co-transcribed genes (*pslA* to *pslO*, PA2231-2245) which encodes for proteins to synthesize Psl. Psl was found to contain a repeating pentasaccharide consisting of D-mannose, D-glucose, and L-rhamnose (Byrd et al. 2009). Pel polysaccharide is a glucose-rich and cellulase-sensitive extracellular substance which is synthesized by the products of the *pel* gene cluster (*pelA-F*, PA3058-PA3064) (Cruz 2014). Alginate is the exopolysaccharide that is mainly produced by *P. aeruginosa* during pathogenicity.

EPS are a complex mixture of biomolecules, consist of proteins, humic-like substances, polysaccharides, uronic acid, nucleic acid, lipids, and glycoproteins, surrounding the bacterial cells (Sheng et al. 2010). The major functional groups of

EPS can be identified by Fourier transform infrared (FTIR) spectroscopy. These substances contain ionizable functional groups such as carboxyl, phosphoric, amine, and hydroxyl groups, which enable EPS to sequester heavy metals (Liu and Fang 2002; Seneviratne et al. 2015). Most of these functional groups are negatively charged at neutral pH and thereby able to form organometallic complexes with multivalent cations through electrostatic attraction, ion exchange, complexation with functional groups of negatively charged, adsorption and precipitation are the mechanisms involved in metal biosorption (Gutnick and Bach 2000; Zhang et al. 2006). These substances thus detoxify metals by complex formation or by forming an effective barrier surrounding the cell (Rajkumar et al. 2010). The FTIR spectral region between 4000 and 400 cm^{-1} holds the major characteristic bands of the various bonds in EPS functional groups.

EPS contain different complexing sites; "hard" (e.g., carboxylic and phenolic) and "soft" (e.g., nitrogen and sulfur-containing) (Zhu et al. 2012). The main electron donor atoms in the EPS are nitrogen in amino-sugars and oxygen in hydroxyl and carboxyl groups. These atoms can easily bind with soft metal cations of strong covalent characteristics (Pb^{2+}, Cu^{2+}, and Zn^{2+}) (Fang et al. 2011). Whereas, other metal cations such as Ni^{2+} and Cd^{2+} form weaker covalent bonds with EPS ligands (Joshi and Juwarkar 2009). The environmental pH also effect on the metal adsorption process by EPS. Metal ion affinity by EPS varies under different conditions, Pb > Cu > Cd at pH 6 and Cu > Pb> Cd at pH 7–8 (Comte et al. 2008).

8.3 Rhizosphere Acidification and Heavy Metal Mobilization

Recent research studies imply that microorganisms could be the key players in heavy metal mobilization. Soil microflora increases the solubility and speciation of heavy metal ions by secretion of various organic ligands and by decomposition of organic matter. Both bacteria and plant roots are able to produce organic acids, such as malate, citrate, gluconate, 2-oxoglutarate, succinate, and oxalate. Thereby it creates a low pH environment in the rhizosphere and enhances the heavy metal mobilization and uptake. As the microbes consume root exudates, they are involved in the production of a wide range of organic acids especially in situations where nutrients may be limiting (Rózycki and Strzelczyk 1986). This could be the reason for higher heavy metal uptake in contaminated sites since most of those sites contain nutrient low degraded soils. It has been reported that LMWOA influence heavy metal speciation and the bioavailability of heavy metals to plants and microorganisms (Renella et al. 2004).

Organic acids can bind metal ions in soil solution by complexation reaction. However, the stability of the ligand-metal complexes is dependent on several factors: number of carboxylic groups and their position, the binding form of the heavy metals, pH of soil solution (Jones 1998). Organic acids released by plant-associated microbes play an important role in the complexation of toxic and essential ions and increase their mobility for plant uptake. It was observed that Zn solubilizing

Gluconacetobacter diazotrophicus produce gluconic acid derivative, 5-ketogluconic acid, which aids in the solubilization of Zn compounds. Similarly, mobilization of Pb and Zn were observed with inoculation of common metal-resistant *Bacillus* strains (Wani et al. 2007). The metal-resistant endophytic bacteria, *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 have also been reported to enhance Pb accumulation in *Brassica napus* through excretion of organic acid. The organic acid producing fungi, *Aspergillus niger* was able to mobilize large amounts of Pb and P from pyromorphite indicated the presence of organic acid in dissolution of minerals (Sheng et al. 2008).

The role of organic acids, acetic and malic acids was exhibited in a study stimulating Cd uptake by maize roots and reported that the organic acid with low stability constant was able to enhance large amount of Cd accumulation in maize (Han et al. 2006). On the other hand, some studies have reported either no effect or negative effects of organic acids in heavy metal mobilization (Braud et al. 2006). The inoculation of organic acid producing bacteria *Bacillus subtilis* in metal contaminated agriculture soils showed no significant effect on the mobilization of Cr and Pb (Braud et al. 2006).

Organic acids function as natural chelating agents which are capable of solubilizing heavy metals from soil (Wasay et al. 1998). *Pseudomonas fluorescens* is a very important rhizobacterium in rhizosphere (Rodríguez and Fraga 1999; Sivasakthi et al. 2013). It produces a variety of organic acids for various functions in the rhizosphere. The gluconic acid production in fluorescent pseudomonads is catalyzed by membrane-bound glucose dehydrogenase (Gcd). In many Gram-negative bacteria, the synthesis of gluconic acid is dependent on pyrroloquinoline quinone (PQQ) as an enzymatic cofactor of the Gcd (de Werra et al. 2009). Biosynthesis of citric acid, which is also an effective organic acid involves condensation of oxaloacetate (OAA) and acetyl-CoA catalyzed by the enzyme citrate synthase.

8.4 Siderosphore Production by Bacteria

Siderosphores are low-molecular-weight (>10 kDa) iron chelating compounds that are mainly produced under low Fe conditions by bacteria, fungi, and plants to facilitate uptake of iron (Chu et al. 2010; Hider and Kong 2010). Siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. Whereas they can also form stable complexes with other heavy metals (Glick and Bashan 1997; Rajkumar et al. 2010). Hence, enhance their bioavailability in the rhizosphere. Binding of the siderophore to a metal increases the soluble metal concentration. Screening with 16 different metals (Ag^{+}, Al^{3+}, Cd^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+}, Eu^{3+}, Ga^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, Tb^{3+}, Tl^{+}, and Zn^{2+}) it is revealed that pyoverdine siderophores produced by *Pseudomonas aeruginosa* are able to chelate all these metals (Braud et al. 2009a).

Table 8.2 Siderophore production by rhizospheric bacteria

Bacterial sp.	Metal	Plant	Reference
Streptomyces tendae	Cd	*Helianthus annuus*	Dimkpa et al. (2009a)
Pseudomonas eruginosa	Pb, Cr	*Zea mays*	Braud et al. (2009b)
Bacillus sp. SLS18	Mn, Cd	*Sorghum bicolor*	Luo et al. (2012)
Psychrobacter Bacillus weihenstephanensis Bacillus cereus	Ni	*Alyssum serpyllifolium and Phleum phleoides*	Ma et al. (2009)
Pseudomonas putida	Pb, Cd	*Vigna radiata*	Tripathi et al. (2005)

About 500 different siderophores have been identified. Even though they differ in overall structure, the functional groups that coordinate Fe are not diverse. They are broadly classified into three main groups based on the chemical nature of the moieties donating the oxygen ligands for Fe(III) coordination, classified as hydroxycarboxylate, catecholate, or hydroxamate type siderophores (Raymond and Dertz 2004). The biosynthetic pathways of siderophores are tightly connected to aerobic metabolism involving molecular oxygen and acids (citrate, succinate, and acetate) originating from the final oxidation of the citric acid cycle.

The production of siderophores in the rhizosphere involves in growth-promoting effect of bacteria on plants (Braud et al. 2009b). The role of SPB in metal uptake of hyperaccumulator plants are extensively studied (Table 8.2). Siderophores produced by soil microbes play an important role in complexing toxic metals and radionuclides and in increasing their mobility in soils. Metal-resistant SPB can increase the efficiency of phytoextraction directly by enhancing the metal accumulation in plant tissues (Dimkpa et al. 2009b; Rajkumar et al. 2010). Siderophores produced by rhizosphere bacteria solubilize unavailable forms of heavy metal-bearing minerals by complexation (Braud et al. 2009b). Plants can then uptake metals from metal–siderophore complexes possibly by root-mediated processes, such as chelate degradation and release of metals, the direct uptake of siderophore–metal complexes or by a ligand exchange reaction. The production of siderophores has also been demonstrated in some mycorrhizal fungi also. It has been reported that the ectomycorrhizal fungi (EMF), *Scleroderma verrucosum, Suillus luteus,* and *Rhizopogon luteolus* produce catecholates and hydroxamates siderophores under iron-deficient conditions (Goodell et al. 1997; Machuca et al. 2007). It is suggested that siderophore-producing microbes are possible to improve heavy metal uptake in plants. However, the mechanisms essential for the plant metal uptake through microbial siderophore-mediated processes are still under research.

Table 8.3 Biosurfactant production by bacteria

Class of the surfactant	Microorganism	References
Rhamnolipids	*Pseudomonas aeruginosa*, *Acinetobacter, Enterobacter*	Toribio et al. (2010), Hošková et al. (2013)
Trehalolipids	*Mycobacterium tuberculosis*, *Rhodococcus erythropolis*, *Arthrobacter* sp.	Kuyukina and Ivshina (2010), Shao (2011), Desai and Banat (1997)
Sophorolipids	*Torulopsis bombicola*	Inoue and Ito (1982)
Corynomycolic acid	*Corynebacterium lepus*	Cooper et al. (1979, 1981a)
Fatty acids, phospholipids, and neutral lipids	*Rhodococcus erythropolis, Acinetobacter* sp.	Rahman and Gakpe (2008)
Surfactin	*Bacillus subtilis*	Cooper et al. (1981b)
Lichenysin	*Bacillus licheniformis*	Yakimov et al. (1996)
Polymeric biosurfactants (emulsan, alasan, lipomanan liposan)	*Acinetobacter calcoaceticus, Candida lipolytica*	Rubinovitz et al. (1982), Rufino et al. (2007)

8.5 Biosurfactants

Biosurfactants are microbial metabolites that facilitate the metal mobilization and improve phytoremediation is microbially produced. These are microbial compounds that demonstrate high surface activity and emulsifying activity. They are amphiphilic molecules with a nonpolar (hydrophobic) tail and a polar/ionic (hydrophilic) head. A hydrophilic group consists of mono-, oligo-, or polysaccharides, peptides or proteins, and a hydrophobic moiety usually contains saturated, unsaturated, and hydroxylated fatty acids or fatty alcohols (Lang 2002). Biosurfactants are categorized as glycolipids, lipopeptides, phospholipids, fatty acids, and neutral lipids. They are either anionic or neutral (Mulligan et al. 2001).

The microorganisms produce low- and high-molecular-weight biosurfactants. The low-molecular-weight types are generally glycolipids or lipopeptides. The glycolipids include trehalose tetraesters, dicorynomycolates, fructose lipids, sophorolipids, and rhamnolipids. Lipopeptides include surfactin, viscosin, and polymixin (Table 8.3).

The role of surfactants possesses different mechanisms in metal removal process. According to the le Chatelier's Principle, metals in a nonionic form can complex with biosurfactants and removed from the surface. Cationic surfactants also have the ability to reduce the association of metals by competition (Herman et al. 1995). The biosurfactants produced by microbes form complexes with heavy metals at the soil interface, desorbs metals from soil matrix, thus increasing metal solubility and bioavailability in the soil solution. Soil type, soil pH, cation exchange capacity (CEC), and particle size also influence biosurfactant action. They act as a soil washing agent due to their ability to solubilize metals within their micellae.

Due to their anionic nature, low toxicity, biodegradability, and excellent surface-active properties of biosurfactants are used in heavy metal removal in soil. The ability of biosurfactants to remove heavy metals from an oil-contaminated soil was demonstrated by batch washes with surfactin, a rhamnolipid, and a sophorolipid, respectively, by *Bacillus subtilis, Pseudornonas aeruginosa,* and *Tomlopsis bornhicola* (Mulligan et al. 1999), whereas they observed surfactin or rhamnolipid could remove the organically bound copper and that the sophorolipid with acid could remove the carbonate and oxide-bound zinc.

8.6 Metal Reduction and Oxidation

Oxidation or reduction reactions are also involved to alter the bioavailability of heavy metals in the plant microbial system. Metal oxidation by rhizosphere microbes is also an interesting and an important process for phytoextraction process. Sulfur-oxidizing bacteria was found to be better than acid treatment for heavy metal solubilization in sulfidic municipal sludges (Blais et al. 1992). A study compared the leaching potential of indigenous sulfur-oxidizing bacteria with acid treatment and they concluded that indigenous sulfur-oxidizing bacteria can be used for heavy metal mobilization (Seidel et al. 1998).

Iron- and sulfur-oxidizing bacteria, *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, respectively, were enriched from contaminated soil and were able to leach >50 % of the metals present (As, Cd, Co, Cu, Ni, V, Zn, B, and Be) (Gomez and Bosecker 1999). It was observed that sulfur-oxidizing rhizosphere bacteria are able to enhance Cu mobilization in contaminated soils and its uptake in plant tissue (Shi et al. 2011). The authors showed that the sulfur-oxidizing bacteria are able to reduce the rhizospheric soil pH by the conversion of reducing sulfur to sulfates, thus enhances the Cu availability for plant uptake. Applications of sulphur-oxidizing bacteria in combination with mychorhizal infection resulted in a significant additive effect on root's Cd uptake and root bioaccumulation (Khorrami Vafa et al. 2012). While (Yang et al. 2012) reported that addition of the As-reducing bacteria promoted the growth of *P. vittata*, it has increased As accumulation (44 %), activated soil insoluble As, and reduced As leaching compared to the untreated control.

The synergistic interaction of metal-oxidizing and -reducing microbes on heavy metal mobilization in contaminated soils has also been studied. The co-inoculation of Fe-reducing bacteria and the Fe/S-oxidizing bacteria significantly increased the mobility of Cu, Cd, Hg, and Zn by 90 % (Beolchini et al. 2009).

8.7 Stress Reduction

The phytoremediation process is also dependent on the plant's ability to tolerate heavy metal toxicity and also to yield a certain biomass. Heavy metals (HMs) are toxic for plant growth development and reproduction. It is one of the major abiotic

stresses that cause detrimental effects to the plants growth. The redox-active HMs are directly involved in the redox reactions in cells and result in the reactive oxygen species. Redox-inactive HMs also results in oxidative stress through indirect mechanisms such as interaction with the antioxidant defense system, disruption of the enzymatic reactions, or induction of lipid peroxidation. The result of HM toxicity is the excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG), both of which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, and DNA damages in plants. The oxidative stress causes discolouration of leaves, deformation of leaves, growth retardation, leaf curling, and disorders in physiological and biochemical reactions. Thereby, it reduces the accumulation of sufficient biomass of the pollutant.

Production of ethylene is one major signal molecule that induces the stress effects that enhances senescence. The endogenous production of ethylene is enhanced significantly, and it causes harmful effects on root growth and thus the growth of whole plant. Certain plant growth-promoting rhizobacteria (PGPR) contain the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which regulates ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into alpha-ketobutyrate and ammonia (Saleem et al. 2007). Inoculation of plants with rhizobacteria containing ACC deaminase activity or transgenic plants expressing ACC deaminase genes produce longer roots and greater root density.

Most of the ACC are synthesized by plant roots, which are subjected to hydrolysis by ACC deaminase bacteria to produce ammonia and a-ketobutyrate. Microbes are able to uptake and then hydrolyze the ACC, and thereby it results subsequent reduction of ACC amount outside the plant. To maintain the equilibrium between the internal and external ACC levels, plants secrete ACC from inner tissues into the rhizosphere. Rhizobacteria containing ACC deaminase activity stimulates ACC exudation from plant roots. And consumption of ACC by microorganisms, which acts as a source of carbon and nitrogen is a driving force for ACC secretion by the plant. The growth of microorganisms containing ACC deaminase is more prominent in the rhizosphere compared to the bulk soil. And consumption of ACC by microorganisms, acts as a driving force for ACC secretion by the plant. As a result, it decreases the level of ACC within the plant, which leads to a reduction in the endogenous ethylene biosynthesis (Glick et al. 1998). The stress reduction in plants cause elimination in root inhibition and enhance root growth which cause an enhanced uptake of inorganic contaminants. It has been reported that due to the inoculation of ACC deaminase-producing heavy metal-resistant bacterium, Kluyvera ascorbata was able to protect canola (Brassica napus) and tomato (Lycopersicon esculentum) seeds from the toxicity of high concentrations of nickel chloride ($NiCl_2$) grown under gnotobiotic conditions (Burd et al. 1998). They ascribed this effect to the ability of the bacterium to lower the level of stress ethylene induced by Ni.

8.8 Plant Growth Promotion

It has been known that 80 % of microorganisms that isolated from the rhizosphere of various crops possess the ability to synthesize and release plant growth regulators (Patten and Glick 1996). IAA is the major plant growth regulator synthesized by the microorganisms. The endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria (Arshad et al. 2007; Glick 2012). IAA stimulates seed and tuber germination, increases the root development, and initiates lateral and adventitious root formation and helps to develop resistance to stressful conditions in plants. IAA produced by rhizobacteria interferes with the above ground physiological processes of plants by changing the plant auxin pool. Whereas bacterial IAA increases root surface area and length, and thus increase the soil nutrient uptake (Glick 2012).

It has been well documented that there are at least five different pathways for the synthesis of IAA (Spaepen and Vanderleyden 2011; Patten and Glick 1996). (1) IAA formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in a majority of bacteria, namely, *Erwinia herbicola*, *Pseudomonas*, *Bradyrhizobium, Rhizobium, Azospirillum, Klebsiella*, and *Enterobacter*, (2) The conversion of tryptophan into indole-3-acetic aldehyde may involve an alternative pathway, which is found in pseudomonads and azospirilla, (3) IAA biosynthesis via indole-3-acetamide formation, which is reported for phytopathogenic bacteria *Agrobacterium tumefaciens, Pseudomonas syringae*, and *E. herbicola,* (4) IAA biosynthesis that involves tryptophan conversion into indole-3-acetonitrile is found in the cyanobacterium (*Synechocystis* sp.), and (5) the tryptophan-independent pathway which is more common in plants and cyanobacteria (Ahemad and Kibret 2014).

8.9 Future Perspectives

Even though phytoremediation is a very important aspect in pollution remediation, the role of rhizospheric organisms is very poorly understood. In several studies, it has been revealed that inoculation of heavy metal-resistant microbes is able to enhance the metal uptake. However, further studies should be conducted to determine the mechanisms behind their role.

- The role of siderophores in plant heavy metal uptake has been discussed in many research studies, but there are no exact mechanisms that have developed to understand the role of siderophores in plant metal uptake and more research towards siderophore interactions with heavy metals will add knowledge into science.
- Only a few researches have focused on the production of low-molecular-weight organic acids and their role in rhizosphere acidification. Further studies may enhance the understanding how the low-molecular-weight organic acids increase

rhizosphere acidity to dissolve more metals into the soil solution. In this case, the monoprotic, diprotic, and triprotic organic acids may need more attention to observe their role in reducing pH of the soil solution.

- Use of biosurfactants in removing organic pollutants have been investigated well in recent years; however, it has not been investigated against heavy metals. Therefore, more attention towards this may increase the potential of biosurfactants in the field of environmental sciences.
- Many laboratory and pilot scale studies already showed that rhizoremediation can contribute to the restoration of polluted sites. However, a selection of suitable rhizoremediation system consisting of a plant inoculated with a bacterium or a consortium with degradation capacity has not been investigated thoroughly.
- Interaction of multiple metals with multiple plants and microorganisms is also an aspect which needs further attention as in the actual field various different types of microorganisms or heavy metals may hinder the rhizoremediation. Heavy metals in diverse valance forms, anionic and cationic forms behave in a different way. Depending on various conditions such as microorganisms present, their ability to secrete EPS, biosurfactants, pH of the rhizosphere, etc., the rhizoremediation capacity may vary. This needs further studies.
- The microbial processes can enhance phytoextraction either by increasing the solubility of the metals in the soil through the production of siderophore, organic acid or biosurfactant or promote plant growth via the IAA or ACC deaminase or the release of growth-limiting nutrients from the soil. However, only a modest attention has been given to elucidate the processes that have the greatest impact on phytoextraction efficiency. More research on these areas will generate new information and knowledge.
- Studies using molecular and biotechnological advances towards biosurfactants, siderophores, plant growth promoters, etc. may yield useful novel information where engineered systems can be constructed for phytoremediation where artificially altered microbial functions supports plant growth promotion and phytoextraction. Such advanced systems will be an interesting tool to further improve and develop bioremediation.

References

Ahemad M, Kibret M (2013) Recent trends in microbial biosorption of heavy metals: a review. Biochem Mol Biol 1:19–26

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 25:356–362

Beolchini F, Dell'Anno A, De Propris L, Ubaldini S, Cerrone F, Danovaro R (2009) Auto-and heterotrophic acidophilic bacteria enhance the bioremediation efficiency of sediments contaminated by heavy metals. Chemosphere 74:1321–1326

Bitton G, Freihofer V (1977) Influence of extracellular polysaccharides on the toxicity of copper and cadmium toward *Klebsiella aerogenes*. Microb Ecol 4:119–125

Blais J, Tyagi R, Auclair J, Huang C (1992) Comparison of acid and microbial leaching for metal removal from municipal sludge. Water Sci Technol 26:197–206

Braud A, Jézéquel K, Vieille E, Tritter A, Lebeau T (2006) Changes in extractability of Cr and Pb in a polycontaminated soil after bioaugmentation with microbial producers of biosurfactants, organic acids and siderophores. Water Air Soil Pollut Focus 6:261–279

Braud A, Hannauer M, Mislin GL, Schalk IJ (2009a) The *Pseudomonas aeruginosa* pyochelin-iron uptake pathway and its metal specificity. J Bacteriol 191:3517–3525

Braud A, Jézéquel K, Bazot S, Lebeau T (2009b) Enhanced phytoextraction of an agricultural Cr-and Pb-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere 74:280–286

Burd GI, Dixon DG, Glick BR (1998) A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Byrd MS, Sadovskaya I, Vinogradov E, Lu H, Sprinkle AB, Richardson SH, Ma L, Ralston B, Parsek MR, Anderson EM (2009) Genetic and biochemical analyses of the *Pseudomonas aeruginosa* Psl exopolysaccharide reveal overlapping roles for polysaccharide synthesis enzymes in Psl and LPS production. Mol Microbiol 73:622–638

Chompoothawat N, Wongthanate J, Ussawarujikulchai A, Prapagdee B (2010) Removal of cadmium ion from aqueous solution by exopolysaccharide-producing bacterium, *Ralstonia* sp. Fresenius Environ Bull 19:2919–2923

Chu BC, Garcia-Herrero A, Johanson TH, Krewulak KD, Lau CK, Peacock RS, Slavinskaya Z, Vogel HJ (2010) Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. Biometals 23:601–611

Comte S, Guibaud G, Baudu M (2008) Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. J Hazard Mater 151:185–193

Cooper DG, Zajic JE, Gracey D (1979) Analysis of corynomycolic acids and other fatty acids produced by *Corynebacterium lepus* grown on kerosene. J Bacteriol 137:795–801

Cooper D, Zajic J, Denis C (1981a) Surface active properties of a biosurfactant from *Corynebacterium lepus*. J Am Oil Chem Soc 58:77–80

Cooper D, Macdonald C, Duff S, Kosaric N (1981b) Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. Appl Environ Microbiol 42:408–412

Cruz KA (2014) Extracellular polysaccharides production by bacteria as a mechanism of mercury tolerance. Rutgers University-Graduate School, New Brunswick

Czaczyk K, Myszka K (2007) Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation. Polish J Environ Stud 16:799

de Souza MP, Chu D, Zhao M, Zayed AM, Ruzin SE, Schichnes D, Terry N (1999) Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. Plant Physiol 119:565–574

de Werra P, Péchy-Tarr M, Keel C, Maurhofer M (2009) Role of gluconic acid production in the regulation of biocontrol traits of *Pseudomonas Fluorescens* CHA0. Appl Environ Microbiol 75:4162–4174

Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 61:47–64

Dimkpa C, Merten D, Svatoš A, Büchel G, Kothe E (2009a) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696

Dimkpa CO, Merten D, Svatoš A, Büchel G, Kothe E (2009b) Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biol Biochem 41:154–162

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890

Fang L, Wei X, Cai P, Huang Q, Chen H, Liang W, Rong X (2011) Role of extracellular polymeric substances in Cu (II) adsorption on *Bacillus subtilis* and *Pseudomonas putida*. Bioresour Technol 102:1137–1141

Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: the "house of biofilm cells". J Bacteriol 189:7945–7947

Garbisu C, Alkorta I (2003) Basic concepts on heavy metal soil bioremediation. Eur J Min Proc Environ Protect 3:58–66

Glazebrook J, Reed JW, Reuber TL, Walker GC (1990) Genetic analyses of *Rhizobium meliloti* exopolysaccharides. Int J Biol Macromol 12:67–70

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15:353–378

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68

Gomez C, Bosecker K (1999) Leaching heavy metals from contaminated soil by using *Thiobacillus ferrooxidans or Thiobacillus thiooxidans*. Geomicrobiol J 16:233–244

Goodell B, Jellison J, Liu J, Daniel G, Paszczynski A, Fekete F, Krishnamurthy S, Jun L, Xu G (1997) Low molecular weight chelators and phenolic compounds isolated from wood decay fungi and their role in the fungal biodegradation of wood. J Biotechnol 53:133–162

Gupta R, Schuster M (2012) Quorum sensing modulates colony morphology through alkyl quinolones in *Pseudomonas aeruginosa*. BMC Microbiol 12:30

Gutnick D, Bach H (2000) Engineering bacterial biopolymers for the biosorption of heavy metals; new products and novel formulations. Appl Microbiol Biotechnol 54:451–460

Han F, Shan X, Zhang S, Wen B, Owens G (2006) Enhanced cadmium accumulation in maize roots—the impact of organic acids. Plant Soil 289:355–368

Hao X, Mohamad OA, Xie P, Rensing C, Wei G (2014) Removal of zinc from aqueous solution by metal resistant symbiotic bacterium *Mesorhizobium amorphae*. Sep Sci Technol 49:376–387

Hattori H (1996) Decomposition of organic matter with previous cadmium adsorption in soils. Soil Sci Plant Nutr 42:745–752

Herman DC, Artiola JF, Miller RM (1995) Removal of cadmium, lead, and zinc from soil by a rhamnolipid biosurfactant. Environ Sci Technol 29:2280–2285

Hider RC, Kong X (2010) Chemistry and biology of siderophores. Nat Prod Rep 27:637–657

Hošková M, Schreiberová O, Ježdík R, Chudoba J, Masák J, Sigler K, Řezanka T (2013) Characterization of rhamnolipids produced by non-pathogenic Acinetobacter and Enterobacter bacteria. Bioresour Technol 130:510–516

Inoue S, Ito S (1982) Sophorolipids from Torulopsis bombicola as microbial surfactants in alkane fermentations. Biotechnol Lett 4:3–8

Iyer A, Mody K, Jha B (2004) Accumulation of hexavalent chromium by an exopolysaccharide producing marine *Enterobacter cloaceae*. Mar Pollut Bull 49:974–977

Jang A, Kim S, Kim S, Lee S, Kim IS (2001) Effect of heavy metals (Cu, Pb, and Ni) on the compositions of EPS in biofilms. Water Sci Technol 43:41–48

Jones DL (1998) Organic acids in the rhizosphere—a critical review. Plant Soil 205:25–44

Joshi PM, Juwarkar AA (2009) In vivo studies to elucidate the role of extracellular polymeric substances from Azotobacter in immobilization of heavy metals. Environ Sci Technol 43:5884–5889

Khan A, Kuek C, Chaudhry T, Khoo C, Hayes W (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Khorrami Vafa M, Shokri K, Sayyadian K, Rejali F (2012) Contribution of microbial associations to the cadmium uptake by peppermint (Mentha piperita). Ann Biol Res 3:2325–2329

Kidambi SP, Sundin GW, Palmer DA, Chakrabarty AM, Bender CL (1995) Copper as a signal for alginate synthesis in *Pseudomonas syringae* pv. syringae. Appl Environ Microbiol 61: 2172–2179

Kunito T, Saeki K, Nagaoka K, Oyaizu H, Matsumoto S (2001) Characterization of copper-resistant bacterial community in rhizosphere of highly copper-contaminated soil. Eur J Soil Biol 37:95–102

Kuyukina MS, Ivshina IB (2010) Rhodococcus biosurfactants: biosynthesis, properties, and potential applications. Biology of *Rhodococcus*. Springer, Berlin, pp. 291–313

Lang S (2002) Biological amphiphiles (microbial biosurfactants). Curr Opin Colloid Interface Sci 7:12–20

Lau T, Wu X, Chua H, Qian P, Wong P (2005) Effect of exopolysaccharides on the adsorption of metal ions by *Pseudomonas* sp. CU-1. Water Sci Technol 52:63–68

Leigh JA, Walker GC (1994) Exopolysaccharides of *Rhizobium*: synthesis, regulation and symbiotic function. Trends Genet 10:63–67

Liu H, Fang HH (2002) Characterization of electrostatic binding sites of extracellular polymers by linear programming analysis of titration data. Biotechnol Bioeng 80:806–811

Luo S, Xu T, Chen L, Chen J, Rao C, Xiao X, Wan Y, Zeng G, Long F, Liu C (2012) Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. SLS18. Appl Microbiol Biotechnol 93:1745–1753

Ma Y, Rajkumar M, Freitas H (2009) Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. J Hazard Mater 166:1154–1161

Machuca A, Pereira G, Aguiar A, Milagres A (2007) Metal-chelating compounds produced by ectomycorrhizal fungi collected from pine plantations. Lett Appl Microbiol 44:7–12

Marques AP, Rangel AO, Castro PM (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Technol 39:622–654

Masák J, Čejková A, Schreiberová O, Řezanka T (2014) Pseudomonas biofilms: possibilities of their control. FEMS Microbiol Ecol 89:1–14

Mulligan CN, Yong RN, Gibbs BF (1999) On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil. Environ Prog 18:50–54

Mulligan C, Yong R, Gibbs B (2001) Surfactant-enhanced remediation of contaminated soil: a review. Eng Geol 60:371–380

Pal A, Paul A (2013) Optimization of cultural conditions for production of extracellular polymeric substances (EPS) by serpentine rhizobacterium *Cupriavidus pauculus* KPS 201. J Polym 2013:692374

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Pérez JAM, García-Ribera R, Quesada T, Aguilera M, Ramos-Cormenzana A, Monteoliva-Sánchez M (2008) Biosorption of heavy metals by the exopolysaccharide produced by *Paenibacillus jamilae*. World J Microbiol Biotechnol 24:2699–2704

Pulsawat W, Leksawasdi N, Rogers P, Foster L (2003) Anions effects on biosorption of Mn (II) by extracellular polymeric substance (EPS) from *Rhizobium etli*. Biotechnol Lett 25:1267–1270

Puyen ZM, Villagrasa E, Maldonado J, Diestra E, Esteve I, Solé A (2012) Biosorption of lead and copper by heavy-metal tolerant *Micrococcus luteus* DE2008. Bioresour Technol 126:233–237

Rahman PK, Gakpe E (2008) Production, characterisation and applications of biosurfactants—review. Biotechnology 7(2):360–370

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149

Raskin I, Kumar PN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290

Raymond K, Dertz E (2004) Biochemical and physical properties of siderophores. Iron transport in bacteria. ASM Press, Washington, DC, pp. 3–17

Renella G, Landi L, Nannipieri P (2004) Degradation of low molecular weight organic acids complexed twith heavy metals in soil. Geoderma 122:311–315

Reuber T, Reed J, Glazebrook J, Glucksmann M, Ahmann D, Marra A, Walker G (1991) *Rhizobium meliloti* exopolysaccharides: genetic analyses and symbiotic importance. Biochem Soc Trans 19:636–641

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rozycki H, Strzelczyk E (1986) Organic acids production by *Streptomyces* spp. isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). Plant and Soil 96:337–345

Rubinovitz C, Gutnick D, Rosenberg E (1982) Emulsan production by *Acinetobacter calcoaceticus* in the presence of chloramphenicol. J Bacteriol 152:126–132

Rufino R, Sarubbo L, Campos-Takaki G (2007) Enhancement of stability of biosurfactant produced by *Candida lipolytica* using industrial residue as substrate. World J Microbiol Biotechnol 23:729–734

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Salehizadeh H, Shojaosadati S (2003) Removal of metal ions from aqueous solution by polysaccharide produced from Bacillus firmus. Water Res 37:4231–4235

Seidel H, Ondruschka J, Morgenstem P, Stottmeister U (1998) Bioleaching of heavy metals from contaminated aquatic sediments using indigenous sulfur-oxidizing bacteria: a feasibility study. Water Sci Technol 37:387–394

Seneviratne M, Seneviratne G, Madawala H, Iqbal M, Rajakaruna N, Bandara T, Vithanage M (2015) A preliminary study of the role of bacterial–fungal co-inoculation on heavy metal phytotoxicity in serpentine soil. Aust J Bot 63:261–268

Shao Z (2011) Trehalolipids, biosurfactants. Springer, New York, pp. 121–143

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156:1164–1170

Sheng GP, Yu HQ, Li XY (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol Adv 28:882–894

Shi JY, Lin HR, Yuan XF, Chen XC, Shen CF, Chen YX (2011) Enhancement of copper availability and microbial community changes in rice rhizospheres affected by sulfur. Molecules 16: 1409–1417

Sivasakthi S, Kanchana D, Usharani G, Saranraj P (2013) Production of plant growth promoting substance by *Pseudomonas fluorescens* and *Bacillus subtilis* isolated from paddy rhizosphere soil of Cuddalore district, Tamil Nadu, India. Int J Microbiol Res 4:227–233

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harbor Perspect Biol 3:a001438

Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9

Teitzel GM, Parsek MR (2003) Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa. Appl Environ Microbiol 69:2313–2320

Toribio J, Escalante AE, Soberón-Chávez G (2010) Rhamnolipids: production in bacteria other than *Pseudomonas aeruginosa*. Eur J Lipid Sci Technol 112:1082–1087

Tripathi M, Munot HP, Shouche Y, Meyer JM, Goel R (2005) Isolation and functional characterization of siderophore-producing lead-and cadmium-resistant *Pseudomonas putida* KNP9. Curr Microbiol 50:233–237

Ueshima M, Ginn BR, Haack EA, Szymanowski JE, Fein JB (2008) Cd adsorption onto *Pseudomonas putida* in the presence and absence of extracellular polymeric substances. Geochim Cosmochim Acta 72:5885–5895

Vu B, Chen M, Crawford RJ, Ivanova EP (2009) Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14:2535–2554

Wani PA, Khan MS, Zaidi A (2007) Chromium reduction, plant growth–promoting potentials, and metal solubilizatrion by *Bacillus* sp. isolated from alluvial soil. Curr Microbiol 54:237–243

Wei Q, Ma LZ (2013) Biofilm matrix and its regulation in *Pseudomonas aeruginosa*. Int J Mol Sci 14:20983–21005

Whiting SN, de Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. Environ SciTechnol 35:3144–3150

Wilson AR, Lion LW, Nelson YM, Shuler ML, Ghiorse WC (2001) The effects of pH and surface composition on Pb adsorption to natural freshwater biofilms. Environ Sci Technol 35: 3182–3189

Yakimov MM, Fredrickson HL, Timmis KN (1996) Effect of heterogeneity of hydrophobic moieties on surface activity of lichenysin A, a lipopeptide biosurfactant from *Bacillus licheniformis* BAS50. Biotechnol Appl Biochem 23:13–18

Yang Q, Tu S, Wang G, Liao X, Yan X (2012) Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by *Pteris vittata* L. Int J Phytoremediation 14:89–99

Zhang D, Wang J, Pan X (2006) Cadmium sorption by EPSs produced by anaerobic sludge under sulfate-reducing conditions. J Hazard Mater 138:589–593

Zhu L, Dai X, Yu YW, Qi HY, Xu XY (2012) Role and significance of extracellular polymeric substances on the property of aerobic granule. Bioresour Technol 107:46–54

Chapter 9
Role of Biosurfactants on Microbial Degradation of Oil-Contaminated Soils

Sandamali Wijesekara, Mihiri Seneviratne, and Meththika Vithanage

Abstract Hydrocarbon contamination of soil is one of the major environmental problems today due to activities related to the petrochemical industry. Mechanical and chemical remediation and restoration to remove hydrocarbons from contaminated sites have limited effectiveness and costly. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. Fungi and bacteria have been considered as highly effective in oil degradation. Several bacteria are even known to feed exclusively on hydrocarbons; Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and Rhodococcus. Fungal genera, namely, Amorphoteca, Neosartorya, Talaromyces, and Graphium are proved to be the potential organisms for hydrocarbon degradation. Although laboratory experiments have indicated that the bacteria can ubiquitously degrade oil constituents, to date there is little convincing evidence that bioaugmentation (addition of more bacteria) significantly enhances the extent of oil biodegradation in soil. The potential benefits of using genetically modified bacteria represent a research frontier with significant results. However, many concerns are often raising due to the effectiveness of indigenous species, limited understanding of various phytoremediation mechanisms, including the regulation of enzyme systems that degrade pollutants. Thus, this chapter presents an updated overview of petroleum hydrocarbon degradation by microorganisms focusing biosurfactants and their mechanisms.

Keywords Oil pollution • Biodegradation • Petroleum hydrocarbons • Bioaugmentation • Phytoremediation

S. Wijesekara • M. Seneviratne
Department of Botany, Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka

M. Vithanage (✉)
Chemical and Environmental Systems Modeling Research Group,
National Institute of Fundamental Studies, Kandy, Sri Lanka
e-mail: meththikavithanage@gmail.com

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_9

9.1 Introduction

Oil hydrocarbon pollution originates from both natural and anthropogenic activities. Contamination due to exploration, draining off from drill sites, accidental spillage, refinery, and transportation has led to global environmental problems (Das and Chandran 2010). The physical and biological effects of oil in the aquatic environment include reduction of light transmission, reduction of dissolved oxygen, damage to water birds, and smothering of intertidal organisms. The toxic effects are exhibited mostly by the light portion (low boiling) of the oil due to the presence of poly aromatic hydrocarbon (PAH) (Atlas and Bartha 1992). The effects include cell damages and death of sensitive organisms and larval stages. The mitigation of oil pollution in sea, soil, and aquatic environment is a difficult task, and various efforts have been taken by the international community to curtail this problem.

In large-scale accidents, oil release into the environment harms the biological system due to the biomagnification of toxic compounds with toxic elements via food webs and food chains (Dillard et al. 1997; Head et al. 2006). The degradation of oil hydrocarbon in aquatic environment, sludge, soil, sediment, and costal ecosystem with natural phenomenon is great deal to overcome these types of problems arising due to the oil contamination. The xenobiotic nature of the oil products and its derivatives are high-risk factors of the environmental degradation due to the bioaccumulation (Head et al. 2006). Oil consists of different compositional ingredients with different chemical and physical properties (Hasanuzzaman et al. 2007). Once petroleum oil is spilled and leaked over the aquatic masses, it will disperse through the surface while releasing volatile compounds to atmosphere (Garrett et al. 2003).

Some hydrocarbons, such as PAH, are known as potent mutagens and carcinogens that pose serious human and environmental health risks. Therefore, the removal of hydrocarbons is essential to improve environmental health. Many different treatment techniques have been studied in order to restore and rehabilitate the soil. The main remediation methods practiced are containment, thermal desorption, incineration, and microbial degradation (Henner et al. 1997).

Cleanup technologies such as physical and chemical methods, volatilization, photooxidation, chemical oxidation, and bioaccumulation seem to be rarely successful on petroleum hydrocarbons due to the recalcitrant nature and not cost-effective when compared to microbial bioremediation (Prince 1997). Oil pollutants are slow in degradation under normal conditions due to the functional groups present and low water solubility (Desai et al. 2012; Hassanshahian et al. 2012). Therefore, it needs special specific conditions to enhance the degradation of particular oil pollutant at a considerable rate and has to reduce time taken to clean up the contaminated site. Degradation of oil depends on the nature of crude oil, composition, and genes responsible for the secretion of enzymes and ecological and environmental factors. Apart from that, efficiency of oil bioavailability is restricted by poor water solubility of oil contaminant (Banat et al. 2010). However, the mechanical removal of

oily substances is very expensive and makes more harmful effects on animals and environment during the period of time (Urum et al. 2006). Recent studies have focused on bioremediation through the indigenous microorganisms in the oil-contaminated surroundings as a potential remediation measure. This chapter provides the background and updated information on microbial degradation of petroleum hydrocarbon contaminants towards the better understanding in bioremediation challenges.

9.2 Bioremediation of Oil Contaminants

Bacteria have long been considered as one of the predominant hydrocarbon-degrading agents found in the environment. The success of bioremediation technologies applied to hydrocarbon-polluted environments depends highly on the biodegrading capabilities of native microbial populations or exogenous microorganisms used as inoculants (Al-Wasify and Hamed 2014; Marchant and Banat 2012). Bioremediation is defined as the use of microorganisms in the biological system to detoxify or remove pollutants using their diverse metabolic capabilities. This process uses an evolving method for the removal and degradation of many environmental pollutants including the products of the petroleum industry (Tang et al. 2011).

Microbial biodegradation is cost-effective, and it serves as highly efficient alternative compared to many types of approaches (Koutinas et al. 2007). Enhancement of crude oil degradation with the use of microorganisms has been identified as a novel approach to overcome oil contamination (Banat et al. 2010). Therefore, many studies have focused on the enhancement of biodegradation of oil contaminants to recover the oil pollution through bioremediation process to clean up the contaminated sites not cleaning up (Soberón-Chávez and Maier 2011). On the other hand, only few studies have concentrated on the generation of the toxic compounds during the bioremediation process by microorganism in some special remediation systems such as salt marshes and estuarine pools (Middaugh et al. 1998; Shelton et al. 1999). The efficiency of microbial degradation has been revealed by with the aerobic degradation (Das and Chandran 2010). According to the studies carried out to reveal the biodegradation of oil, it has aimed to isolate and identify the bacteria and other organisms having highly adapted nature to the contaminated sites to degrade contaminants (Zajic et al. 1977). Scientists have observed and reported isolated organisms from study sites which were chemically and physically stable and their metabolic activities are optimum at the particular environment (Zhang et al. 2010).

Recent researches have paid their attention on natural, safe, cost-effective materials to treat oil-contaminated sites (Van Hamme et al. 2003). Simple oil contaminants with simple structures are processed with different metabolic pathways based on the unique enzyme systems present in microorganisms (Van Hamme et al. 2003). Therefore, they have chosen biosurfactant as the best potential method of

remediation of oil spills (Wei et al. 2005). Development of the possible eco-friendly tool to handle the contaminated sites using efficient strategies and an applicable model system is important (Franzetti et al. 2010a). Therefore it has been discovered that the use of naturally occurring bacteria for removal of oil contaminants have built up the new interpretations for the oil recoveries with the production of biosurfactants (Ron and Rosenberg 2002).

Biodegradation of oil can be influenced by the microorganisms present in the natural environment as well as by introducing improved microbial cultures (Table 9.1). Oil spill bioremediation has two main approaches: (a) bioaugmentation, where known oil-degrading bacteria are added to supplement the existing microbial population, and (b) biostimulation, where the growth of indigenous oil degraders are stimulated by the addition of nutrients or other growth-limiting substrates with the supplement of enriching factors of medium (Ojo 2005). Oil-degrading microbial consortia are used as the improved cultures, and it consists with one or more microorganisms showing an ability to degrade oil at a considerable rate (Rosenberg and Ron 1999). Several studies have proven with the evidence that the use of microorganisms to oil recovery is a desirable alternative to remove the oily contaminants from the environment (Banat et al. 2000) (Table 1).

Microorganisms should possess special metabolic activities responsible for the production of metabolites to utilize the oily compounds. Therefore, specific biochemical reactions of microorganisms are needed for the expression of the production of metabolites for the degradation of total petroleum products (Atlas and Bartha 1992). It is very important to know that the reasonable performance of microorganism during biodegradation depends on the enzymatic activity and its mechanism (Obayori et al. 2009).

But if an unexpectable oil drainage occurred, the naturally occurring microbial consortium is unable to degrade the spilled oil totally at considerable rate, because an already existing small proportion of microorganism consortium may not be enough to degrade the bulk of oil contaminant and some of them may inactive due to the toxicity nature of oil to them (Shelton et al. 1999). Therefore, it is necessary to use the best biotechnological approach to overcome this type of problems before dispersing oil over the surface. To fulfill this purpose, selection of the best biotechnological solution is very important as an innovative technique.

Table 9.1 Biological systems used to recover the oil contaminations in several countries

Country	Biological system used	References
India, Goan coast	*P. aeruginosa*, *Escherichia fergusonii*	Pasumarthi et al. (2013)
Egypt, Gemsa Bay	*P. xanthomarina*, *P. stutzeri ATCC 17588*	El-Sheshtawy et al. (2014)
China, Nanjing Refinery sludge	*Pseudomonas* sp.	Gao et al. (2000)
Northeast India	*Bacillus subtilis P. aeruginosa* sp.	Das and Mukherjee (2007)
Taiwan	*P. aeruginosa EM1*	Wu et al. (2008)
China—oil storage tank	*Pseudomonas* sp.	Yan et al. (2012)

Exxon Valdez and BP Deepwater Horizon oil spill were the two large-scale oil spills (Atlas and Hazen 2011). The experimental evidence suggests that those are the worst oil spills in US history with svere environmental impacts of oil contamination (Bragg et al. 1994). Because petroleum hydrocarbons consist in crude oil with diverse derivatives of both aromatic and aliphatic hydrocarbons, is easily escape into groundwater reservoirs and surrounding environment. Generally petroleum hydrocarbons naturally exist in all marine environments with the compatible nature of naturally occurring microorganisms. Those microorganisms are capable of utilizing those contaminants by using oil as an energy source (Garon et al. 2002).

9.3 Mechanism of Microbial-Mediated Petroleum Hydrocarbon Degradation

Basically bioremediation expresses the mean of transformation and mineralization of toxic compounds via the endogenous or exogenous process of microorganisms (Franzetti et al. 2010b). In endogenous activity, carbon source has to go inside before it undergoes to the transformation event. Compared to endogenous process, exogenous process occurred in external environment. Therefore, it is essential to bind with carbon and the microbial cell before utilizing it (Mulligan and Gibbs 2004). The ability to form biosurfactants is found in numerous bacterial and fungal species (Desai and Banat 1997), for instance, *Pseudomonas* sp., *Rhodococcus* sp., *Acinetobacter* sp., *Bacillus* sp., *Achromobacter* sp., *Brevibacillus* sp., *Lysinibacillus* sp., *Alcaligenes* sp., *Candida* sp., and *Torulopsis* sp. (Desai and Banat 1997). Biosurfactants enables them to grow on hydrophobic substrate. However, due to low solubility of petroleum hydrocarbon, the biodegradation of contaminant by microorganisms is highly restricted (Banat et al. 2010), but with the presence of extracellular biosurfactants, these compounds are solubilized, increasing its bioavailability to microorganisms by providing contaminant as an effective energy source for them (Joshi et al. 2008). Studies on biosurfactants showed the degradation of contaminant depends on the length and nature of arrangement of the carbon chain. Aliphatic moieties are easily degradable than the rest of aromatic carbon structures (Setti et al. 1993), because aromatic fraction is a heavily branched compound and it is difficult to degrade. Hence, aromatic fraction may persist in the environment for a longer period without any change and will pose a significant harmful effect on an environment (Hasanuzzaman et al. 2007).

Most of the recent studies were focused on the degradation of crude oil. Diverse groups of microorganisms are able to synthesize various types of secondary metabolites (Banat 1995), which have an ability to produce biosurfactant effects to degrade pollutants in an efficient way. Especially the biosurfactants are commonly employed in crude oil recovery and have been explained (Banat et al. 2000).

9.4 Structure of the Biosurfactant

Biosurfactants are the naturally occurring structurally diverse groups showing surface-active mechanisms with amphiphilic properties. It consists with both hydrophobic and hydrophilic moieties with surface-active compounds (Fig. 9.1). Most biosurfactants are anionic or neutral, but few are cationic due to the presence of amine group. Biosurfactants are amphipathic molecules which accumulate at interfaces, decrease interfacial tensions, and form aggregates such as micelles. However, biosurfactants show unique biochemical properties which are highly important in biological applications. Biosurfactants are easily accessible and acceptable for the environmental application, especially for both land and sea (Cameotra et al. 2010).

9.5 Classification of Biosurfactants

Biosurfactants are classified based on their chemical structure, composition, molecular weight, physicochemical properties, mode of action, and the microbial origin that produced them (Zinjarde and Pant 2002) (Table 9.2). Its hydrophobic fraction may consist with the saturated or unsaturated fatty acid, and hydrophilic fraction may consist with the amino acids, peptides, and saccharides. Based on its chemical composition and chemical behavior, it can be divided into three major classes as follows (Desai and Banat 1997):

1. Glycolipids (lipopeptides, lipoproteins, and phospholipids)
2. Polymeric biosurfactants
3. Particulate biosurfactants

In some cases, biosurfactants can be categorized based on its molecular weight as low- and high-molecular-weighted polymers (Rosenberg and Ron 1999). Low-molecular-mass biosurfactants include glycolipids, phospholipids, and lipopeptides, whereas high-molecular-mass surfactants include amphipathic polysaccharides, lipopolysaccharides, proteins, lipoproteins, and complex mixtures of

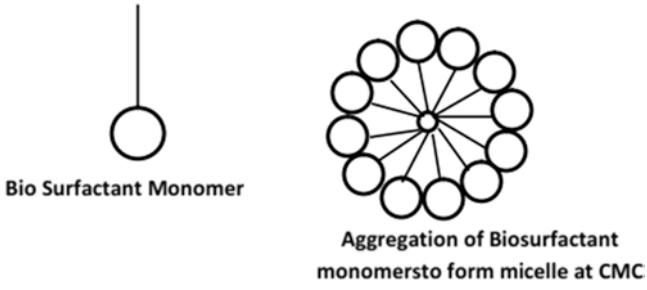


Fig. 9.1 Surfactant structure showing hydrophobic head and hydrophilic tail and formation of micelle at CMC

Table 9.2 Classification of biosurfactants and their use in remediation of oil contaminants

Biosurfactant		Microorganisms	References
Group	Class		
Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa*	Rosa et al. (2010)
	Trehalolipids	*Rhodococcus erythropolis*, *Arthrobacter* sp., *Nocardia* sp.	Franzetti et al. (2010b), Shao (2011)
	Sophorolipids	*Torulopsis* bombicola, *T. petrophilum*	Whang et al. (2008)
Fatty acids phospholipids and neutral lipids	Corynomycolic acid	*Corynebacterium lupus*	Bozo-Hurtado et al. (2012)
	Spiculisporic acid	*Penicillium spiculisporum*	Tabuchi et al. (1977)
	Phosphatidylethanolamine	*Pseudomonas fluorescens*	Appanna et al. (1995)
Lipopeptides	Surfactin	*Bacillus subtilis*	Cooper et al. (1981)

biopolymers. Compared to the high-molecular-mass biosurfactants, low-molecular-weight compounds are efficient in lowering the surface and interfacial tension of contaminant, whereas high-molecular-mass compounds are effective at stabilizing the oil in water emulsion (Calvo et al. 2009).

9.6 Properties of Biosurfactants: Natural Choice for Bioremediation with Biosurfactants

Biosurfactants have been identified as eco-friendly, cost-effective, easily degradable substrate produce by microorganisms as their secondary metabolite, and they are producing mixture of biopolymers (Rosenberg and Ron 1999). It aims to mineralize and/or biotransform toxic contaminants to nontoxic or low-toxic compounds while utilizing it by microorganisms (Sanscartier et al. 2009). Due to high demand for the biosurfactants, the attention has been paid on artificially synthesizing this eco-friendly natural compound (Atlas and Bartha 1992). However, it has shown that synthetic chemicals had severe adverse effects on an environment over the use of natural surfactants. When the biosurfactant is ready to activate on any hydrophobic substrate to degrade it, biosurfactant accumulates at the interface between fluid and solid with the effective reduction of surface and interfacial tension. Hence, it is allowing those two dissimilar phases to mix and interact with each other more easily (Soberón-Chávez and Maier 2011).

Measurement of biosurfactant activity has been done by the measurement of surface and interfacial tension, stabilization of emulsion, and hydrophilic-lipophilic

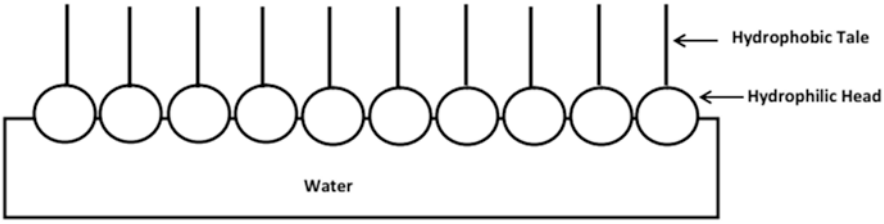


Fig. 9.2 Accumulation of biosurfactants at the interface

balance (HLB) of the surfactants and contaminant (Oberbremer et al. 1990). Surface tension was measured at the air-water and oil/water interface with the use of tensiometer (Salamanca et al. 2001). The high molecular weight surfactants are less effective in reducing interfacial tension, but are important at coating the oil droplets and preventing their coalescence (Fig. 9.2). These are highly efficient emulsifiers that work at low concentrations (Ron and Rosenberg 2002). Emulsification activity is measured by emulsification assay. It is based on the ability of the surfactant to regenerate droplets in particular aqueous assay system (Kim et al. 2000). Biosurfactants can increase the bound substrates by desorbing them from surfaces or by increasing their apparent water solubility.

9.7 Relationship Between Surface Tension and Critical Micelle Concentration (CMC)

The efficiency and effectiveness of biosurfactant depends on the formation of critical micelle concentration of the surface-active compound with the collection of sufficient surface-active molecules on the substrate. At the concentration above CMC, biosurfactant molecules gathered to form micelles, vesicles, and lamellae on the substrate as a continuous bilayer (Whang et al. 2008). Formation of micelle enables to increase the solubility and bioavailability on hydrophobic contaminant to make easy degradation while reducing the surface tension (Desai and Banat 1997). However, an efficient biosurfactant has the lower CMC value; therefore, less amount of biosurfactant is required to decrease the surface tension (Nguyen et al. 2008) (Fig. 9.3). It has direct relationship with the formation of microemulsion layer (Desai and Banat 1997). That allows the contaminant to adjust to the hydrophobic environment leading the formation of stable liquid mixture of water and oily particles by the formation of droplets dispersed on the liquid phase (Soberón-Chávez and Maier 2011).

Relationship between surface tension and CMC is expressed in the semilogarithmic plot of the surface tension of a solution against the surfactant concentration as mentioned below (Fig. 9.4). HLB indicates the promotion of surfactant activity in water-in-oil or oil-in-water to emulsify the contaminant easier. If HLB value is less

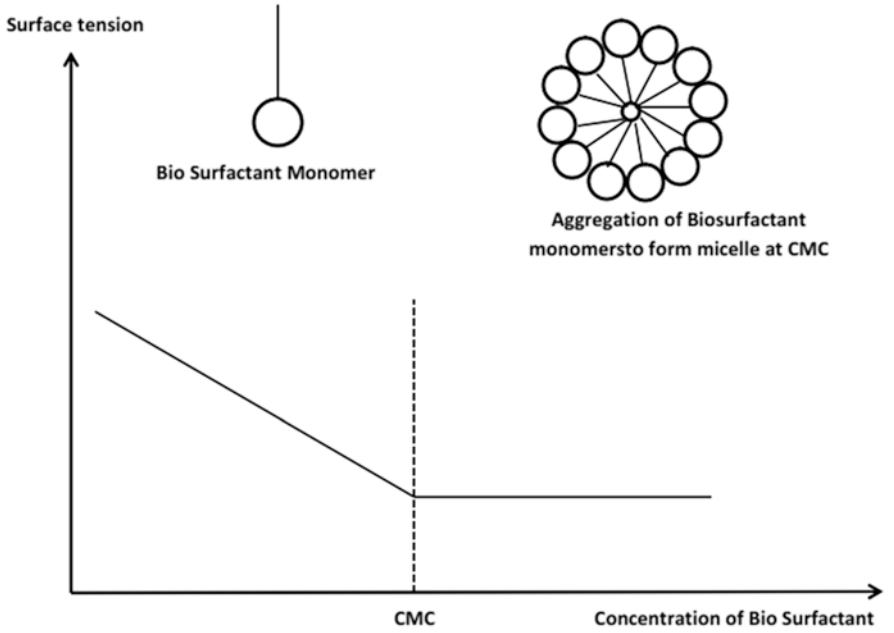


Fig. 9.3 Surface tension vs. concentration of purified biosurfactant

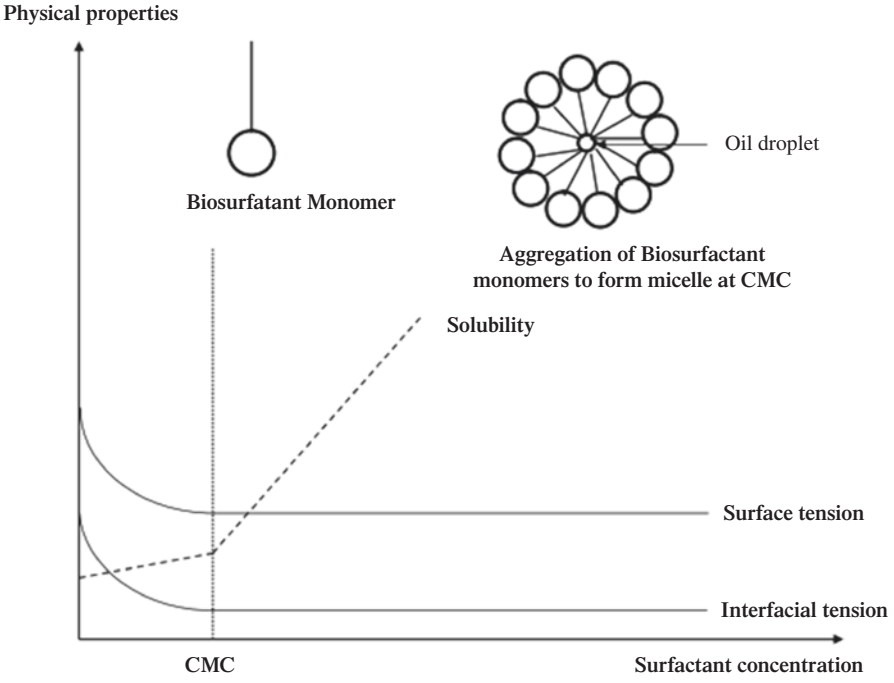


Fig. 9.4 The relationship between biosurfactant concentration, surface tension, and formation of micelles

than 6, it is preferred to emulsify water-in-oil; if the HLB value is in between 10–18, it favors the emulsification of contaminant in oil-in-water (Oberbremer et al. 1990).

In practical approaches, HLB value can be measured as follows:

HLB value = 20 * Mh/M.
Mh—molecular weight of the hydrophilic fraction
M—molecular weight of the whole molecule

HLB value of 0 corresponds to a completely lipophilic/hydrophobic molecule, and a value of 20 corresponds to a completely hydrophilic/lipophobic molecule. Therefore, HLB values of surfactants are useful in predicting the surfactant properties of the surface-active compounds.

If the two moieties of biosurfactant are not arranged in an equal way, this formula does not apply (Vollbrecht et al. 1999).

Ex-HLB value of glycolipid in the system containing water and hydrophobic phase consists with soybean and cyclohexane mixture. For this system, HLB value is measured as follows:

$A = (HLB_{needed} - HLB_B)/(HLB_A - HLB_B)$
A = % of cyclohexane
HLB_A—HLB of cyclohexane (−15)
HLB_B—HLB of soybean (−6)

Using this formula, most stable emulsification index can be determined for the selected system.

9.8 Effect of pH and Temperature on Biosurfactant Stability

The activity of biosurfactant decreases at extreme pH values (Champion et al. 1995). However, biosurfactants are stable at high temperatures (Banat 1995). Biosurfactant was stable during exposure to high salinity (10 % NaCl), elevated temperatures (120 °C for 15 min), and within a wide pH range (4.0–10.0) (Shavandi et al. 2011). The activity of the biosurfactant was enhanced optimally at NaCl concentration of 5 %, pH of 8.0, and temperature of 40 °C. The biosurfactant retained 77 % of its original activity after 120 min of exposure to heat at a temperature of 100 °C (Ilori et al. 2005).

9.9 Mechanisms of Biosurfactants in Biodegradation

The use of biosurfactants to remove oil contaminants is a promising method that can improve the effectiveness of bioremediation in contaminated environments. They can enhance hydrocarbon bioremediation by two mechanisms. The first mechanism

includes the induction of substrate bioavailability for microorganisms, while the other involves interaction with the cell surface which increases the hydrophobicity of the surface allowing hydrophobic substrates to associate more easily with bacterial cells (Mulligan and Gibbs 2004). By reducing surface and interfacial tensions, biosurfactants increase the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons. Addition of biosurfactants to the polluted site through the process of bioaugmentation is capable of degrading contaminants. This process is expected to enhance the hydrocarbon biodegradation by mobilization, solubilization, or emulsification of contaminants efficiently (Nguyen et al. 2008).

Usually microbial aerobic degradation of alkanes follows intracellular enzymatic pathway (Morgan and Watkinson 1990). Aerobic degradation is the most rapid and complete degradation of organic pollutants by microorganisms (Fig. 9.5). Organic pollutants including various oil pollutants and its derivatives are initially attacked by microorganisms through an oxidative process. Hereafter, activation and incorporation of pollutant into cell is essential before utilizing it. Those two processes are catalyzed by two enzymes named as oxygenase and peroxidase. Then intermediates are formed via the peripheral degradation pathway, Eg–TCA cycle (Fig. 9.6).

Instead of oxygenase degradation pathway being used for the degradation of oil derivatives, it is linked with the many other enzymatic pathways. The activity of cytochrome P450 alkane hydroxylase enzyme is the well-studied enzyme on this event (van Beilen and Funhoff 2007). Degradation depends on the change of length, and the enzyme system efficiency is more important to initiate the degradation (Table 9.3).

Apart from that, another enzyme system has been discussed by van Beilen and Funhoff (2005). The enzyme is di-iron methane monooxygenase showing membrane-bound copper-containing monooxygenase responsible for the degradation of oil with elementary constituent in toxic level (van Beilen and Funhoff 2007).

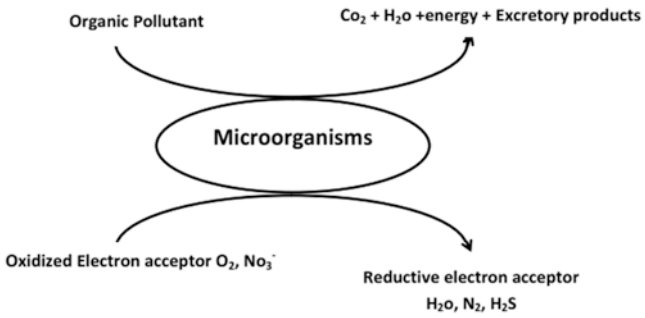


Fig. 9.5 Oxidative biodegradation and its mechanism

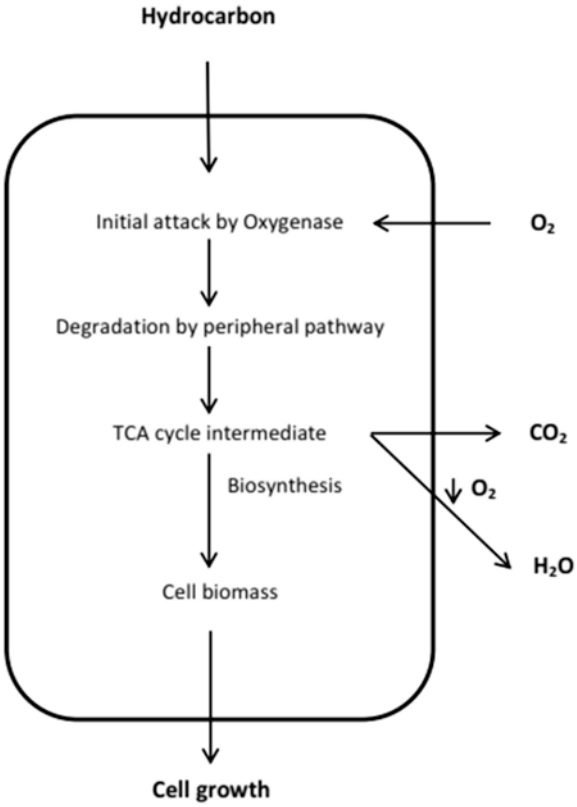


Fig. 9.6 Participation of an enzyme system for the degradation of contaminant

Table 9.3 Enzymes involve in biodegradation of oil contaminants

Enzyme	Substrate	Organisms	References
Methane monooxygenase	Short alkanes	*Methylococcus* sp.	Shigematsu et al. (1999)
	Cyclohexane	*Methylomonas* sp.	Shigematsu et al. (1999)
Eukaryotic P450	Fatty acids, C_{10}–C_{15} alkanes	*Candida bombicola*	Van Bogaert et al. (2009)
Bacterial P450	C_5–C_{16}	*Acinetobacter* sp., *Mycobacterium* sp.	Fujii et al. (2006)
Dioxygenase	C_{10}–C_{30}	*Acinetobacter* sp.	Bundy et al. (1998)
Alkane hydroxylase	C_5–C_{16}	*Pseudomonas* sp., *Rhodococcus* sp.	Whyte et al. (2002)

9.10 Attachment of Microorganism to Substrate as a Mechanism to Obtain the Degradation of Contaminant

In the latest literature, it has explained the interaction between microorganisms and hydrocarbon which is used to utilize as a substrate by microorganisms (Van Hamme et al. 2003). However, by modulating of cell surface, hydrophobicity may increase the surface permeability allowing microorganisms to make direct contact with the oil drops (Patist et al. 2000). However, researchers have reported the low hydrophobicity modulation by microorganism on cell surface permits their adhesion to micelles of emulsified oil. Based on their studies, pseudo-solubilization of substrate by microorganism was used to make direct contact of microbial cells with large oil droplets. Also it has been revealed that at the different growth stages of microorganisms, their capability of producing different secondary metabolites has an ability with changing hydrocarbon accession modes (Singh et al. 2006). An increment of bioavailability of oil to the bacterial has been exhibited by the biosurfactant-coated contaminant (Cameotra and Makkar 2004). This process is involving internalization of hydrocarbon inside the cell for subsequent degradation. It is similar in the process called active pinocytosis (Cameotra and Singh 2009).

9.11 Conclusions and Future Perspectives

Continuous release of oil pollutants through either natural or industrial processes is occurring as one of the leading environmental problems. Hence, the bioremediation as a green technology is an acceptable solution due to the cost and effort involved. Therefore, the use of biology systems is playing an important role in this novel strategy. Hence, currently the demand for biological system in various industries is steadily increasing. In this case, biosurfactants play an important role on oil hydrocarbon degradation. However, the commercial success of biosurfactant is still limited because it has to pay high production cost. It is necessary to find out more economically feasible methods to produce biosurfactant in large scale. Hence, future research must focus on producing low-cost biosurfactants and test. Application of biosurfactant and other microbial degradation methods is still in under experimental level. Therefore, the use of these experimental processes in the field scale is important in order to validate the laboratory results. Most of the studies carried out under in vitro condition are needed to move to field scale evaluating its effectiveness.

The development of microbial degradation of contaminant with improvements in genetic expression via the manipulation of gene expression is very important. Application of this technology benefits in large-scale application of microbial degradation and provides us with a cleaner environment in a safe way. The genetically modified (GM) bacteria give a novel approach in biotechnology. However, still

there is a limited understanding on their enzyme regulation systems that degrade pollutants. Studies must focus on degrading both aliphatic and aromatic compounds effectively at the same time, and in this case, the biotechnology may play a rigorous role. Thus, the integration of biological engineering and biotechnology is possible and profoundly needed in order to achieve high yields and low costs on biosurfactant production. High-throughput analysis, predictive computational modeling, or simulation and experimental perturbation can be combined to generate new knowledge in order to design strategies for efficient reactions.

References

Al-Wasify RS, Hamed SR (2014) Bacterial biodegradation of crude oil using local isolates. Int J Bacteriol 2014:863272

Appanna VD, Finn H, Pierre MS (1995) Exocellular phosphatidylethanolamine production and multiple-metal tolerance in *Pseudomonas fluorescens*. FEMS Microbiol Lett 131:53–56

Atlas RM, Bartha R (1992) Hydrocarbon biodegradation and oil spill bioremediation. Adv Microb Ecol 12:287–338

Atlas RM, Hazen TC (2011) Oil biodegradation and bioremediation: a tale of the two worst spills in US history. Environ Sci Technol 45:6709–6715

Banat IM (1995) Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. Bioresour Technol 51:1–12

Banat IM, Makkar RS, Cameotra S (2000) Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 53:495–508

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87:427–444

Bozo-Hurtado L, Rocha CA, Malavé R, Suárez P (2012) Biosurfactant production by marine bacterial isolates from the Venezuelan Atlantic Front. Bull Environ Contam Toxicol 89: 1068–1072

Bragg JR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 368:413–418

Bundy BM, Campbell AL, Neidle EL (1998) Similarities between the antABC-encoded anthranilate dioxygenase and the benABC-encoded benzoate dioxygenase of *Acinetobacter* sp. strain ADP1. J Bacteriol 180:4466–4474

Calvo C, Manzanera M, Silva-Castro G, Uad I, González-López J (2009) Application of bioemulsifiers in soil oil bioremediation processes: future prospects. Sci Total Environ 407: 3634–3640

Cameotra SS, Makkar RS (2004) Recent applications of biosurfactants as biological and immunological molecules. Curr Opin Microbiol 7:262–266

Cameotra SS, Singh P (2009) Synthesis of rhamnolipid biosurfactant and mode of hexadecane uptake by *Pseudomonas* species. Microb Cell Fact 8:16

Cameotra SS, Makkar RS, Kaur J, Mehta S (2010) Synthesis of biosurfactants and their advantages to microorganisms and mankind. In: Sen R (ed) Biosurfactants. Springer, New York, pp 261–280

Champion JT, Gilkey JC, Lamparski H, Retterer J, Miller RM (1995) Electron microscopy of rhamnolipid (biosurfactant) morphology: effects of pH, cadmium, and octadecane. J Colloid Interface Sci 170:569–574

Cooper D, Macdonald C, Duff S, Kosaric N (1981) Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. Appl Environ Microbiol 42:408–412

Das K, Mukherjee AK (2007) Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresour Technol 98:1339–1345

Das N, Chandran P (2010) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 2011:1–13

Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential. Microbiol Mole Biol Rev 61:47–64

Desai H, Mehdi TH, Cappello S (2012) Isolation and characterization of two crude oil-degrading yeast strains, *Yarrowia lipolytica* PG-20 and PG-32, from the Persian Gulf. Mar Pollut Bull 64:1386–1391

Dillard LA, Essaid HI, Herkelrath WN (1997) Multiphase flow modeling of a crude-oil spill site with a bimodal permeability distribution. Water Resour Res 33:1617–1632

El-Sheshtawy H, Khalil N, Ahmed W, Abdallah R (2014) Monitoring of oil pollution at Gemsa Bay and bioremediation capacity of bacterial isolates with biosurfactants and nanoparticles. Mar Pollut Bull 87:191–200

Franzetti A, Gandolfi I, Bestetti G, Banat I (2010a) Biosurfactant and bioremediation, successes and failures. In: Plaza G (ed) Trends in bioremediation and phytoremediation. Research Signpost, Kerala, pp 145–156

Franzetti A, Gandolfi I, Bestetti G, Smyth TJ, Banat IM (2010b) Production and applications of trehalose lipid biosurfactants. Eur J Lipid Sci Technol 112:617–627

Fujii T, Narikawa T, Sumisa F, Arisawa A, Takeda K, Kato J (2006) Production of α, ω-alkanediols using Escherichia coli expressing a cytochrome P450 from *Acinetobacter* sp. OC4. Biosci Biotechnol Biochem 70:1379–1385

Gao B, Yang L, Wang X, Zhao J, Sheng G (2000) Influence of modified soils on the removal of diesel fuel oil from water and the growth of oil degradation micro-organism. Chemosphere 41:419–426

Garon D, Krivobok S, Wouessidjewe D, Seigle-Murandi F (2002) Influence of surfactants on solubilization and fungal degradation of fluorene. Chemosphere 47:303–309

Garrett RM, Rothenburger SJ, Prince RC (2003) Biodegradation of fuel oil under laboratory and arctic marine conditions. Spill Sci Technol Bull 8:297–302

Hasanuzzaman M, Ueno A, Ito H, Ito Y, Yamamoto Y, Yumoto I, Okuyama H (2007) Degradation of long-chain n-alkanes (C 36 and C 40) by Pseudomonas aeruginosa strain WatG. Int Biodeterior Biodegrad 59:40–43

Hassanshahian M, Emtiazi G, Cappello S (2012) Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. Mar Pollut Bull 64:7–12

Head IM, Jones DM, Röling WF (2006) Marine microorganisms make a meal of oil. Nature Rev Microbiol 4:173–182

Henner P, Schiavon M, Morel JL, Lichtfouse E (1997) Polycyclic aromatic hydrocarbon (PAH) occurrence and remediation methods. Analusis 25:M56–M59

Ilori M, Amobi C, Odocha A (2005) Factors affecting biosurfactant production by oil degrading *Aeromonas* spp. isolated from a tropical environment. Chemosphere 61:985–992

Joshi S, Bharucha C, Jha S, Yadav S, Nerurkar A, Desai AJ (2008) Biosurfactant production using molasses and whey under thermophilic conditions. Bioresour Technol 99:195–199

Kim SH, Lim EJ, Lee SO, Lee JD, Lee TH (2000) Purification and characterization of biosurfactants from Nocardia sp. L-417. Biotechnol Appl Biochem 31:249–253

Koutinas M, Baptista II, Meniconi A, Peeva LG, Mantalaris A, Castro PM, Livingston AG (2007) The use of an oil–absorber–bioscrubber system during biodegradation of sequentially alternating loadings of 1,2-dichloroethane and fluorobenzene in a waste gas. Chem Engi Sci 62: 5989–6001

Marchant R, Banat IM (2012) Microbial biosurfactants: challenges and opportunities for future exploitation. Trends Biotechnol 30:558–565

Middaugh D, Shelton M, McKenney C Jr, Cherr G, Chapman P, Courtney L (1998) Preliminary observations on responses of embryonic and larval Pacific herring, Clupea pallasi, to neutral fraction biodegradation products of weathered Alaska North Slope oil. Arch Environ Contam Toxicol 34:188–196

Morgan P, Watkinson RJ (1990) Assessment of the potential for in situ biotreatment of hydrocarbon-contaminated soils. Water Sci Technol 22:63–68

Mulligan CN, Gibbs BF (2004) Types, production and applications of biosurfactants. Proc Ind Natl Sci Acad Part B 70:31–56

Nguyen TT, Youssef NH, McInerney MJ, Sabatini DA (2008) Rhamnolipid biosurfactant mixtures for environmental remediation. Water Res 42:1735–1743

Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Omotayo AE, Amund OO (2009) Degradation of hydrocarbons and biosurfactant production by Pseudomonas sp. strain LP1. World J Microbiol Biotechnol 25:1615–1623

Oberbremer A, Müller-Hurtig R, Wagner F (1990) Effect of the addition of microbial surfactants on hydrocarbon degradation in a soil population in a stirred reactor. Appl Microbiol Biotechnol 32:485–489

Ojo O (2005) Petroleum-hydrocarbon utilization by native bacterial population from a wastewater canal Southwest Nigeria. Afr J Biotechnol 5:333–337

Pasumarthi R, Chandrasekaran S, Mutnuri S (2013) Biodegradation of crude oil by *Pseudomonas aeruginosa* and *Escherichia fergusonii* isolated from the Goan coast. Mar Pollut Bull 76: 276–282

Patist A, Bhagwat S, Penfield K, Aikens P, Shah D (2000) On the measurement of critical micelle concentrations of pure and technical-grade nonionic surfactants. J Surfactants Deterg 3:53–58

Prince RC (1997) Bioremediation of marine oil spills. Trends Biotechnol 15:158–160

Ron EZ, Rosenberg E (2002) Biosurfactants and oil bioremediation. Curr Opin Biotechnol 13: 249–252

Rosa CFC, Michelon M, JFdM B, SJ K, CAV B (2010) Production of a rhamnolipid-type biosurfactant by *Pseudomonas aeruginosa* LBM10 grown on glycerol. Afr J Biotechnol 9(53): 9012–9017

Rosenberg E, Ron E (1999) High-and low-molecular-mass microbial surfactants. Appl Microbiol Biotechnol 52:154–162

Salamanca J, Ciampi E, Faux D, Glover P, McDonald P, Routh A, Peters A, Satguru R, Keddie J (2001) Lateral drying in thick films of waterborne colloidal particles. Langmuir 17: 3202–3207

Sanscartier D, Reimer K, Koch I, Laing T, Zeeb B (2009) An investigation of the ability of a 14C-labeled hydrocarbon mineralization test to predict bioremediation of soils contaminated with petroleum hydrocarbons. Bioremediation J 13:92–101

Setti L, Lanzarini G, Pifferi P, Spagna G (1993) Further research into the aerobic degradation of n-alkanes in a heavy oil by a pure culture of a *Pseudomonas* sp. Chemosphere 26:1151–1157

Shao Z (2011) Trehalolipids, biosurfactants. Springer, New York, pp. 121–143

Shavandi M, Mohebali G, Haddadi A, Shakarami H, Nuhi A (2011) Emulsification potential of a newly isolated biosurfactant-producing bacterium, *Rhodococcus* sp. strain TA6. Colloids Surf B: Biointerfaces 82:477–482

Shelton M, Chapman P, Foss S, Fisher W (1999) Degradation of weathered oil by mixed marine bacteria and the toxicity of accumulated water-soluble material to two marine crustacea. Arch Environ Contam Toxicol 36:13–20

Shigematsu T, Hanada S, Eguchi M, Kamagata Y, Kanagawa T, Kurane R (1999) Soluble methane monooxygenase gene clusters from trichloroethylene-degrading Methylomonas sp. strains and detection of methanotrophs during in situ bioremediation. Appl Environ Microbiol 65: 5198–5206

Singh R, Paul D, Jain RK (2006) Biofilms: implications in bioremediation. Trends Microbiol 14:389–397

Soberón-Chávez G, Maier RM (2011) Biosurfactants: a general overview, biosurfactants. Springer, New York, pp. 1–11

Tabuchi T, Nakamura I, Higashi E, Kobayashi H (1977) Factors affecting the production of the open-ring acid of spiculisporic acid by Penicillium spiculisporum. J Ferment Technol 55: 43–49

Tang J, Wang M, Wang F, Sun Q, Zhou Q (2011) Eco-toxicity of petroleum hydrocarbon contaminated soil. J Environ Sci 23:845–851

Urum K, Grigson S, Pekdemir T, McMenamy S (2006) A comparison of the efficiency of different surfactants for removal of crude oil from contaminated soils. Chemosphere 62:1403–1410

Van Beilen JB, Funhoff EG (2005) Expanding the alkane oxygenase toolbox: new enzymes and applications. Curr Opin Biotechnol 16:308–314

Van Beilen JB, Funhoff EG (2007) Alkane hydroxylases involved in microbial alkane degradation. Appl Microbiol Biotechnol 74:13–21

Van Bogaert IN, Demey M, Develter D, Soetaert W, Vandamme EJ (2009) Importance of the cytochrome P450 monooxygenase CYP52 family for the sophorolipid-producing yeast *Candida bombicola*. FEMS Yeast Res 9:87–94

Van Hamme JD, Singh A, Ward OP (2003) Recent advances in petroleum microbiology. Microbiol Mol Biol Rev 67:503–549

Vollbrecht E, Rau U, Lang S (1999) Microbial conversion of vegetable oils into surface-active di-, tri-, and tetrasaccharide lipids (biosurfactants) by the bacterial strain *Tsukamurella* sp. Lipid/Fett 101:389–394

Wei Q, Mather R, Fotheringham A (2005) Oil removal from used sorbents using a biosurfactant. Bioresour Technol 96:331–334

Whang LM, Liu PWG, Ma CC, Cheng SS (2008) Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. J Hazard Mater 151:155–163

Whyte L, Smits T, Labbe D, Witholt B, Greer C, van Beilen J (2002) Gene cloning and characterization of multiple alkane hydroxylase systems in *Rhodococcus* strains Q15 and NRRL B-16531. Appl Environ Microbiol 68:5933–5942

Wu JY, Yeh K, Lu WB, Lin CL, Chang JS (2008) Rhamnolipid production with indigenous *Pseudomonas aeruginosa* EM1 isolated from oil-contaminated site. Bioresour Technol 99: 1157–1164

Yan P, Lu M, Yang Q, Zhang HL, Zhang ZZ, Chen R (2012) Oil recovery from refinery oily sludge using a rhamnolipid biosurfactant-producing *Pseudomonas*. Bioresour Technol 116:24–28

Zajic J, Guignard H, Gerson D (1977) Properties and biodegradation of a bioemulsifier from Corynebacterium hydrocarboclastus. Biotechnol Bioeng 19:1303–1320

Zhang Z, Gai L, Hou Z, Yang C, Ma C, Wang Z, Sun B, He X, Tang H, Xu P (2010) Characterization and biotechnological potential of petroleum-degrading bacteria isolated from oil-contaminated soils. Bioresour Technol 101:8452–8456

Zinjarde SS, Pant A (2002) Emulsifier from a tropical marine yeast, *Yarrowia lipolytica* NCIM 3589. J Basic Microbiol 42:67–73

Chapter 10
Microbial Oxidation of Atmospheric Methane in Natural and Agricultural Upland Soils

Irina K. Kravchenko

Abstract Methane is the second most important greenhouse gas in terms of amounts and effect in the atmosphere. Upland soils of the European Russia are important participants in the global carbon budget, but their role as a sink for atmospheric methane is poorly documented, and little information on biodiversity of methanotrophic microorganisms is available. We have found that managed soils from different climatic regions showed decreased methane oxidation rates in both field and laboratory experiments. Large fluctuations were revealed in CH_4 uptake process at different time scales (monthly, daily, hourly), and soil organic matter, water content, and temperature were seen as the main environmental controlling factors. Methanotrophic populations of unmanaged soils turned out to be much low diverse and dominated by uncultivated methanotrophs. In Podzoluvisol, Luvisol, and Meadow Kastanozem, we have identified deeply branching *pmoA* sequences of *Alphaproteobacteria* referred as NSUC (natural soil uncultivated cluster), formed novel monophyletic cluster with other uncultured methanotrophs. Pronounced shift to cultured methanotrophs was observed in the same soils after agricultural loading.

Keywords Methane uptake • Soils • Aerobic methanotrophs • Land management

10.1 Introduction

Methane (CH_4) is the second most important greenhouse and its increasing concentration in the Earth's atmosphere is linked to today's global warming. Due to changes in human activity and land use, atmospheric methane concentration increased from a preindustrial mixing ratio of about 0.7 ppm to 1.8 ppm currently (Degelmann et al. 2010). Increases in the concentrations of methane are associated with global climate change and net CH_4 flux is controlled by the interplay of biotic

I.K. Kravchenko (✉)
Laboratory of Microbial Survival, Winogradsky Institute of Microbiology,
Research Center of Biotechnology of the Russian Academy of Sciences, 33, bld. 2 Leninsky Ave,
Moscow 119071, Russia
e-mail: irinakravchenko@inbox.ru

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_10

and abiotic factors. Two principal pathways in atmospheric methane elimination are known: photochemical degradation in the stratosphere and microbial oxidation in aerobic, native, and agricultural soils (Bousquet et al. 2006). Taking into account a stratospheric sink of 40 Tg y^{-1} and CH_4 consumption in soils of 30 Tg y^{-1}, the present-day global chemical CH_4 lifetime is estimated to be within the range 8.45 ± 0.38 y^{-1} (Stevenson et al. 2006). The rates of CH_4 consumption in arable soils is generally lower than from native ecosystems under similar environmental conditions and usually are only about 10–30 % of those under forest (Smith et al. 2000). The proportion of biotic atmospheric methane consumption is estimated of 5–15 % in total removal and according to a recent meta-analysis the total annual CH_4 uptake ranges from 12 to 59 Tg CH_4 y^{-1}, with a narrower estimate of 22 ± 12 Tg CH_4 y^{-1} (Dutaur and Verchot 2007).

Many studies have investigated CH_4 uptake in soils of natural ecosystems and have found them to be the sink for atmospheric methane (Conrad and Rothfuss 1991; Börjesson et al. 2001; Suwanwaree and Robertson 2005). Conversion of natural undisturbed soils to arable cropping ecosystems has significantly reduced the CH_4 oxidizing capacity of soils (Le Mer and Roger 2001). Agricultural practices also affect the methanotrophic community structure (Knief et al. 2003; Seghers et al. 2003; Kravchenko et al. 2005). The biological methane oxidation is an important process to minimize global climate change and any negative impact or imbalance may be the reason of the dramatic ecosystem change. Therefore, there is an insistent need of extensive research to study methanotrophic activity in various ecosystems.

Until now, very limited data are available for CH_4 oxidation and consumption in Russian non-wetland, terrestrial soil ecosystems. So, monitoring and process understanding of CH_4 consumption in these soils is required to estimate global greenhouse gases balance and contribution to global warming. This chapter, firstly to our knowledge, synthesizes the environmental and climatic factors influencing the consumption of atmospheric methane as far as diversity of methanotrophic communities in aerated soils of European Russia. We hypothesized the direct connections between the shifts in the microbial communities and the rates of methane fluxes due to the changes of climatic, environmental, and anthropogenic factors.

10.2 CH_4 Fluxes in Upland Soils: Patterns and Environmental Controls

Despite the active study, the ecological representativeness of the data available for methane oxidation in aerated soils remains insufficient. It is especially important to understand the role of the Russian soils, which are usually not included in the general reviews of the atmospheric methane uptake in soils (Smith et al. 2000, 2003; Le Mer and Roger 2001; Dutaur and Verchot 2007; Kirschke et al. 2013; Serrano-Silva et al. 2014) or soil methanotrophs diversity (Aronson et al. 2013) due to the lack of published data. Extremely scarce are studies on methane oxidation that combine field and laboratory methods of soil biogeochemistry and microbiology.

More detailed understanding is needed of the changes in the methane-oxidizing capacity of soils depending on their physicochemical and biological properties that change under the action of ecological and anthropogenic factors.

10.2.1 Methane Uptake in Russian Gray Forest Soil

Since the Russian climate and seasonality is extremely variable, CH_4 consumption is spatially and temporally variable, and temporal variability exists in many scales with different controlling factors. We have chosen gray forest soils (Luvisol in FAO classification) in Moscow Region under different ecosystems (native forest, arable cropping) for detailed study and evaluation of CH_4 fluxes and environmental regulators. Gray forest soils, one of the typical agricultural soils in the European part of Russia, are important participants in the global carbon budget, but their role as a sink for atmospheric methane is poorly documented. We have investigated the variability of CH_4 uptake process and environmental and edaphic characteristics of agricultural gray forest soils, Moscow region, Russia, at different time scales (monthly, daily, hourly). Soil CH_4 uptake was measured using a closed chamber method to quantify soil flux and field data were analyzed in order to elucidate the mechanisms governing short-term and long-term trends and try to illuminate what we miss if we monitor or model this process coarsely in time.

Measurement of in situ surface CH_4 flux at the forest biocenosis and the agrocenosis sites consistently showed atmospheric methane uptake and the magnitude of the flux varying substantially during the annual cycle. The highest methane uptake rates were recorded at growth season (May–September), constituting −0.048 to 0.06 mg C-CH_4 m^{-2} h^{-1} for the forest area, and −0.03 to 0.037 mg C-CH_4 m^{-2} h^{-1} for the agrocenosis. These rates are comparable with that of other terrestrial systems at lower latitudes (−0.1 to −1.0 mg CH_4-C m^{-2} day^{-1}) (Luo et al. 2013). In the cold season (October–April), the capacity of the forest soil for methane oxidation was retained, but its rate decreased. In the agrocenosis soil, methane oxidation in this period reduced significantly and in some cases the methane emission from the soil into the atmosphere was recorded in February and March (Semenov et al. 2004). The average annual rate of methane consumption by the *gray forest* soil in the forest and agrocenosis was evaluated as 0.026 and 0.008 mg C-CH_4 m^{-2} h^{-1} (approximately 2.3 and 0.8 kg of C-CH_4 ha^{-1} y^{-1}), respectively. Average for the 3 year period annual net rate of methane oxidation by gray forest soil in the Moscow region was calculated as 0.02 mg C-CH_4 m^{-2} h^{-1} and aggregated CH_4 sink was 0.68 Gg CH_4 y^{-1} (Semenov et al. 2013).

The pronounced seasonal changes of methane uptake testify to a considerable dependence of the methanotrophic communities on the soil hydrothermal conditions and physicochemical properties. During the observation period, significant changes in the soil temperature and moisture content and slight pH changes were noted. A positive correlation was revealed between the methane oxidation activity and temperature in both forests ($r^2 = 0.735$) and arable ($r^2 = 0.703$) soil. The correlation between the soil moisture content and methane consumption was found to be negative ($r^2 = -0.431$ for the forest soil and $r^2 = -0.634$ for the arable

soil). These values are in good agreements with findings for soils of the Swedish forest biocenosis (Klemedtsson and Klemedtsson 1997).

The significant monthly variations of in situ methane oxidation testified to a considerable dependence of the activity of the microbial community to soil hydrothermal conditions and physicochemical properties. The multiple regression method was applied in order to assess the integral effect of environmental factors on seasonal changes in methane uptake fluxes. The relation between CH_4 uptake fluxes and hydrothermal and physicochemical factors may be described using the following equations:

$$Y1 = 0.257X1 - 0.499X2 + 0.172X3 - 2.168X4 - 10.28X5 - 1.561X6 + 90.8X7 - 363.8 \quad (r^2 = 0.917)$$

$$Y2 = 1.054X1 - 0.128X2 - 0.251X3 - 0.155X4 + 70.86X5 - 22.77X6 + 117.7X7 - 741.3 \quad (r^2 = 0.725)$$

where $Y1$ and $Y2$ are the rates of methane consumption in the soils of the forest and the agrocenosis, respectively, mg C-CH_4 m^{-2} h^{-1}; $X1$ is soil temperature, °C; $X2$ is the moisture content of soil, %; $X3$ is the carbon content of the microbial biomass, mg C per 100 g soil; $X4$ is the nitrogen of the microbial biomass, mg N per 100 g soil; $X5$ is N-NH_4^+ content, mg per 100 g soil; $X6$ is N-NO_3^- content, mg/100 g soil; and $X7$ is pH (Semenov et al. 2004).

The everyday time series of CH_4 fluxes during the snow-free period have revealed wake-like variations daily average fluxes ranged from 2 to 12-fold at 2–4 days apart. Diurnal CH_4 fluxes were determined in summer and autumn. Daily maximum in efflux commonly occurred during the night and large (threefold) diurnal differences were occasionally found. Daytime measurements alone can result in a slight or moderate underestimate of the total CH_4 flux. These diurnal variations were found to be strongly influenced by air temperature, and Q_{10} for methane uptake by surface soil was found to be 2.5±0.5. The diurnal rhythm was similar in microplots with different management and at different seasons, and all of them showed absorption peak at 15–00. On the diurnal uptake basis, the mean uptake at the time interval of 09:00–10:00 may approximately reflect the average status over a day. Based on our data, the average soil fluxes from measurements between 9:00 and 10:00 can be regarded as the representative of daily averages for gray forest soil (Semenov et al. 2013). These findings are in good agreement with data for desert soils in a semiarid region of northern China (Hou et al. 2012). We conclude that diurnal variation in methane fluxes can cause systematic errors in flux estimates, and mean values are used; measurements may result in gross overestimates, underestimates, or even the wrong sign of process.

10.2.2 Methane Uptake in Soils of Different Natural Zones of the European Russia

The East European Plain (also called the Russian Plain) spans over about four million square kilometers and includes a wide diversity of both cultivated and natural landscapes. It lies north and south and consists of gradual changing tundra,

coniferous forest (taiga), mixed and broadleaf forests, grassland (steppe), and semi-desert zones.

Changes in climate, vegetation, soil properties, and nutrition greatly affect biogeochemical cycles of main biogenic elements such as carbon, nitrogen, and phosphorous. Soil zonality is well expressed in the vast plains of Russia covered by homogeneous deposits. Under such conditions, soil follows changes of vegetation formations and temperature/precipitation climate gradients. Therefore, we assessed methane oxidation activity and diversity of aerobic methanotrophic bacteria in nine soil types (both unmanaged and agricultural) geographically distributed across the European part of Russia.

Based on monthly variability patterns of CH_4 uptake in gray forest soil, we have chosen the July–August period for the field studies, and all evaluations were done at the time interval in from 9 to 10 a.m. The soil spectra involved into research is presented in Table 10.1.

It was found that soils of the natural ecosystems, with the exceptions of Solodic Chernozem and Solonetz, showed methane uptake, being, sinks of atmospheric methane. Maximal surface methane oxidation rates were recorded for Podzoluvisol (Podsollivisol on FAO classification) and Kastanozem being 19 and 30 μg CH_4 m^{-2} h^{-1}, respectively. Solodic Chernozem and Solonetz showed positive methane flux up to 6 and 13 μg CH_4 m^{-2} h^{-1}, correspondingly**.** The fluxes of CH_4 uptake rates were within the range reported from other studies in temperate forests in Europe (Smith et al. 2000; Steinkamp et al. 2001; Borken et al. 2003), and in North America (Castro et al. 2000), but lower than fluxes reported in forest studies in Japan (Ishizuka et al. 2000; Tamai et al. 2003) and China (Zhang et al. 2008).

Chernozem from natural steppe ecosystem demonstrated methane uptake flux being 7.6 μg CH_4 m^{-2} h^{-1}, and this finding has confirmed by numerous estimations of atmospheric methane consumption in unmanaged grasslands (Mosier et al. 1991; Zhou et al. 2008; Luo et al. 2013). Our results suggest that some unmanaged steppe biotopes (Kastanozem and Chernozem soils) may also act as natural sinks of atmospheric methane; however, some other unmanaged soils in the steppe region (Solodic Chernozem, Solonetz) may contribute to methane emission to Earth's atmosphere.

Overall, the ability of agricultural soils to oxidize atmospheric methane was three to nine times weaker than in unmanaged soils. Chernozem displayed the least methane uptake being 2.2 μg CH_4 m^{-2} h^{-1}. This trend has been demonstrated previously and, for example, methane oxidation by the soils of natural ecosystems in Iowa, USA, was 0.027–1.046 mg CH_4 m^{-2} day^{-1}, whereas in agricultural ecosystems it constituted 0.077 mg CH_4 m^{-2} day^{-1} and alternated with methane emission (Chan and Parkin 2001). Our results support these observations, and we found agricultural soils to be characterized by decreased methane-consuming ability in comparison to unmanaged ones. The methane uptake fluxes rates are in good agreement with the data obtained by other authors for similar soils under similar ecological conditions. In subarctic and temperate forest soils, the rate of methane oxidation was about 1–3 mg CH_4 m^{-2} day^{-1} and 2.1–6.9 mg CH_4 m^{-2} day^{-1}, correspondingly (Goldman et al. 1995).

Chernozem from natural steppe ecosystem demonstrated methane uptake flux being 7.6 μg CH_4 m^{-2} h^{-1}, and this finding has confirmed by numerous estimations of atmospheric methane consumption in unmanaged grasslands (Mosier et al. 1991;

Table 10.1 Description of environmental sites and selected soil parameters (modified from Kizilova et al. 2013)

Habitat and coordinates	Soil type, FAO	Land management	Vegetation	Selected soil parameters					CH_4 oxidation rate ($\mu g\ CH_4\ m^{-2}\ h^{-1}$)
				Organic C (%)	Total N (%)	C:N	pH (KCl)	$NO_3 + NH_4$, mg/100 g	
Taiga zone Moscow, Puschino (54.50°N, 37.37°E)	Podzoluvisol	Unmanaged	Mixed forest	2.1	0.2	10.5	4.6	0.8	−19.0 ± 3.4
		Managed	Barley field	1.2	0.1	11.9	5.3	0.8	−2.6 ± 1.2
Forest steppe zone Tula, Schekino (54.00°N, 37.31°E)	Luvisol	Unmanaged	Broadleaf forest	1.3	0.1	10.5	4.2	2.3	−26.0 ± 8.1
		Managed	Wheat field	0.9	0.1	9.2	5.1	0.8	−4.3 ± 1.1
Lipetsk, Danki (53.30°N, 38.58°E)	Phaeozem	Unmanaged	Broadleaf forest	4.3	0.3	12.5	5.8	2.6	−19.0 ± 2.0
Steppe zone Voronezh, Bobrov (51.07°N, 40.17°E)	Solodic chernozem	Unmanaged	Birch and aspen forest	2.7	0.2	10.8	6.4	0.9	+6.1 ± 0.4
Voronezh, Talovaya (51.07°N, 40.43°E)	Solonetz	Unmanaged	Grassland	2.9	0.2	11.7	8.2	1.2	+12.9 ± 2.0
Voronezh, Talovaya (51.07°N, 40.43°E)	Chernozem	Unmanaged	Grassland	4.3	0.4	11.5	6.7	2.0	−7.6 ± 2.6
		Managed	Wheat field	3.9	0.3	11.3	6.2	2.0	−2.2 ± 1.2
Dry steppe zone Volgograd, Kachalino (49.49°N, 44.32°E)	Gleyic kastanozem	Unmanaged	Elm and alder forest	3.2	0.3	11.9	6.9	0.8	−24.0 ± 7.0
Volgograd, Ylovlya (49.47°N, 44.31°E)	Kastanozem	Unmanaged	Grassland	1.6	0.1	10.9	5.5	0.7	−30.0 ± 5.2
		Managed	Wheat field	0.9	0.1	9.1	5.8	0.6	−25.0 ± 7.0

Zhou et al. 2008; Luo et al. 2013). Our results suggest that some unmanaged steppe biotopes (Kastanozem and Chernozem soils) may also act as natural sinks of atmospheric methane; however, some other unmanaged soils in the steppe region (Solodic Chernozem, Solonetz) may contribute to methane emission to Earth's atmosphere.

Overall, the ability of agricultural soils to oxidize atmospheric methane was three to nine times weaker than in unmanaged soils. Chernozem displayed the least methane uptake being 2.2 μg CH_4 m^{-2} h^{-1}. This trend has been demonstrated previously and, for example, methane oxidation by the soils of natural ecosystems in Iowa, USA, was 0.027–1.046 mg CH_4 m^{-2} day^{-1}, whereas in agricultural ecosystems it constituted 0.077 mg CH_4 m^{-2} day^{-1} and alternated with methane emission (Chan and Parkin 2001). Our results support these observations, and we found agricultural soils to be characterized by decreased methane-consuming ability in comparison to unmanaged ones. The methane uptake fluxes rates are in good agreement with the data obtained by other authors for similar soils under similar ecological conditions. In subarctic and temperate forest soils, the rate of methane oxidation was equal to 1–3 CH_4 m^{-2} day^{-1} and 2.1–6.9 mg CH_4 m^{-2} day^{-1}, correspondingly (Goldman et al. 1995).

Several factors may have contributed to a drop of the methane oxidation in agricultural ecosystems. First, this is possibly due to the shift from high-affinity methanotrophs to low-affinity methane oxidizers. A similar trend has been reported for Brazilian ferralsols (Dorr et al. 2010). Secondly, extensive NPK and PK fertilizations may inhibit the process of methane oxidation due to competitive and noncompetitive inhibition (Dunfield and Knowles 1995). Some other environmental variables, such as temperature, the water content in soils and carbon supply, can also induce changes in oxidation rates.

10.3 Factors Affecting Atmospheric Methane Oxidation in Soils

Despite numerous investigations aimed to evaluate factors controlling CH_4 oxidation and consumption in aerobic soils, the mechanisms controlling this process are not yet well understood. Based on published evidence and results of our previous studies, it was recognized that CH_4 exchange between soil and atmosphere is affected by different factors including temperature, precipitation, land use, N input, and soil properties, such as soil texture, pH and C/N ratio, and organic matter content. It is difficult to evaluate the influence of a single climate parameter on methane uptake because others often covary or interact. Soil temperature may influence the emission and uptake of the gases through the effects on the activity of microorganisms and roots activity, as long as other factors (water and substrate availability) are not limiting (Meixner and Yang 2006). On the one hand, the water content is important for growth of soil microorganisms (Schindlbacher et al. 2004), and on the other hand influences gas diffusivity (Smith et al. 2003).

Exposure to agricultural practices induces the changes in local environmental conditions and physicochemical and water physical properties of arable soils as compared to intact soils. Based on studies of methane oxidation kinetics, it was hypothesized that the rate of methane oxidation could depend on the microbial community composition (Levine et al. 2011), variations in the processes of carbon and nitrogen mineralization (Von Fischer and Hedin 2007), and the aeration regime (Flechard et al. 2005). At the same time, we are not aware of any experimental studies that experimentally tested these hypotheses. Based on results of our studies on *gray forest* soil, we have assumed that the reduction of methane oxidation activity in arable soil was due to significant and irreversible changes in its physicochemical properties that control this activity.

10.3.1 Temperature

In field studies, the seasonal development of soil temperature usually is reflected in the seasonal course of soil gas emissions. In temperate climates, soil emissions typically peak during summer when temperatures are highest (Priemé and Christensen 1997). In our study of *gray forest* soil, a positive correlation between CH_4 uptake and mean annual air temperature was observed that revealed the importance of soil temperature in interannual variability of methane fluxes (Semenov et al. 2004).

In forest soils, soil temperature was found to be an important controller of CH_4 consumption at temperatures between −5 and 10 °C but had little effect at 10 and 20 °C (Castro et al. 1995). At low temperatures (<10 °C), temperature was found to be stronger modulator than soil moisture for soil CH_4 uptake (Steinkamp et al. 2001). The Q_{10} values for CH_4 oxidation range from 1.1. to 4.8 (De VIisser et al. 2001; Park et al. 2005). These findings are consistent with the results found in temperate forests in Europe (Steinkamp et al. 2001) and in the USA (Castro et al. 1995). It was found that CH_4 consumption increased with rising temperature from 5 to 10°C and remained relatively constant at temperatures between 10 and 20 °C. The optimum for CH_4 oxidation was suggested to be on the interval from 22 to 38 °C (De VIisser et al. 2001).

10.3.2 Soil Moisture

Soil moisture content is one of the main factors limiting the methane oxidation activity affecting diffusion of the gas phase (Striegl 1993) or soil methanotrophs activity by osmotic stress (Schnell and King 1996). Methane oxidation negatively correlated with soil water content due to limited CH_4 diffusion; however, net CH_4 oxidation is possible in wet soil (WFPS > 60 %) due to localized aerobic microsites or anaerobic CH_4 oxidation (Khalil and Baggs 2005). Low soil moisture content also reduces methane oxidation, probably due to stressful moisture deficiency or the accumulation of mineral nitrogen compound species (Steudler et al. 1989; Castro et al. 1995).

It was found that 20 % soil moisture content is the most favorable for methane oxidation in aerated mineral soils (Boeckx and Van Cleemput 1996; Billings et al. 2000). In our study of *gray forest* soil, it was precisely this level of moisture content in situ that corresponded to the periods of the highest methane oxidation activity (Kravchenko et al. 2005). In very dry soils such as in deserts, CH_4 oxidation is higher after precipitation (Strieg et al. 1992) probably because of the osmotic stress. Maximum CH_4 uptake rates were recorded under a diverse range of moisture conditions between 20 and 60 % water-holding capacity (Bowden et al. 1998).

10.3.3 Soil Structure and Porosity

Soil type is strong controlling variable on the water-holding capacity of the soil, and the diffusion of gases into soil has a pronounced effect on CH_4 oxidation. Sandy soil has the low water-holding capacity, and the sand content of temperate grassland has been correlated with CH_4 consumption rates (Born et al. 1990). CH_4 oxidation is associated with air-filled pore space, and soil texture and bulk density are also related to CH_4 oxidation in soil (Boeckx et al. 1997). Soil texture was found to be one of the main factors correlated with CH_4 fluxes, and coarser and medium textured (loam) soils consuming more CH_4 than fine (clay) soils (Dutaur and Verchot 2007). Methane fluxes correlated negatively with bulk density and were very sensitive to cultivation. The low CH_4 uptake rates at the cropland sites and the grassland sites may be influenced by higher bulk densities, which also affect gaseous diffusion (Smith et al. 2003).

The key physical property of soil controlling the gas diffusion and balance between aerobic and anaerobic processes is its porosity. In the *gray forest* soil of native forest, it amounted to 55–57 %, ensuring favorable conditions for water and air permeability. At the agricultural soil, porosity was dropped down to 40–47 %, and the mean pore diameter decreased to 10–100 times (Kravchenko et al. 2005). Additionally, soil aggregates in the agrocenosis soil had low stability and were disintegrated by wetting. These findings are in the agreement with data of MacDonald et al. (1996) and Smith et al. (2003) reported that oxidation is most rapid in coarse-textured forest soils with a well-developed structure We conclude that the predominance of fine and inactive pores and the low water resistance of soil aggregates may be one of the reasons in decrease of the methanotrophic activity in cultivated soil.

10.3.4 Organic Matter Content

Methane cycling in the soil–atmosphere system is determined by the processes of mineralization of organic matter in anaerobic microzones and ascending and descending diffusion in the pore space of the methane produced in the soil and the methane arriving from the atmosphere, as well as by methane oxidation by methane oxidizers. Methane production in aerobic soils is related to the granulometric composition; the presence of large-sized aggregates, inside which anaerobic conditions

may be created; and over consolidation of the upper horizon lower layers, especially in arable soils (Manucharova et al. 2001). In most forest soils, the highest methane oxidation activity was revealed in the upper 4- to 18-cm soil horizon (Adamsen and King 1993; Roslev et al. 1997). The methane produced under this layer is partially or completely oxidized by soil microorganisms. Thus, in aerobic soils, the closed chambers method measures the resultant of the processes, which characterizes the net gas flux and gross methane oxidation.

Organic amendments vary in their effects on CH_4 consumption according to their C:N ratios. For example, adding fresh sugar beet leaves reduced CH_4 consumption to 20 %, whereas adding wheat straw had no effect (Hütsch 1998). Long-term application of organic fertilizers supports the diversity of methanotrophs, as was estimated by group-specific DGGE of 16S rRNA genes (Seghers et al. 2003). Application of bio-based residue (sewage sludge, aquatic plant material, compost, wood material, and compressed beet leaves), added at amounts typical of intensive agricultural practice resulted in significant transient stimulation of methane uptake apparently a result of induced cell-specific activity (Ho et al. 2015).

10.3.5 Nitrogen Compounds

CH_4 fluxes can be altered by N fertilization, but the magnitude and direction of alteration is unclear (Bodelier and Laanbroek 2004; Mohanty et al. 2006; Bodelier 2011). Ammonium fertilization is usually reported to competitively inhibit CH_4 oxidation (Gulledge and Schimel 1998; Bykova et al. 2007; Acton and Baggs 2011). Some studies, however, reported no effect (Tate et al. 2006) or positive promotion (Jacinthe and Lal 2006). Nitrogen fertilization may increase CH_4 uptake or have no effect due to an alleviation of N-limitations of methanotrophs (Bodelier and Laanbroek 2004; Liu and Greaver 2009) on CH_4 fluxes in N-limited environments. Immobilization of mineral N in soils with high cation exchange capacity prevent inhibition of the relatively unspecific particulate methane monooxygenase (pMMO) enzyme of methanotrophs by NH_4^+ (Purkhold et al. 2000; Kravchenko et al. 2002).

These conflicting effects may be due to diversity in methanotrophic communities. It was revealed that application of N fertilizers generally inhibits methane oxidation by type II methanotrophs, but enhances by type I methanotrophs (Mohanty et al. 2006). As such, the coexistence of different methanotrophic communities may reduce the inhibitory effect of NH_4^+ on methane oxidation (Kravchenko et al. 2002). NO_2^- inhibits CH_4 oxidation in arable soils (Kravchenko et al. 2002), but persists in soil only for a few days. The effect of NO_3^- is unclear and varies from no effect (Tate et al. 2006) to negative effect (Wang and Ineson 2003).

A number of investigations have been carried out to evaluate the influence of short-term increased CH_4 and ammonium concentrations on CH_4 oxidation activity (Bender and Conrad 1993; Jensen and Olsen 1998; Tlustos et al. 1998; Kravchenko et al. 2002). However, the response of the microbial community structure to such perturbations is largely unknown.

The data obtained for *gray forest* soil demonstrated a close correlation between methane oxidation and the main processes of nitrogen transformation. The application of $(NH_4)_2SO_4$ at a rate of 60 mg N kg^{-1} resulted in a direct inhibiting of methane oxidation activity of arable *gray forest* soil but had no significant effect on methane oxidation by the forest soil (Kravchenko et al. 2004).

10.4 Methanotrophic Soil Communities in Upland Soils

Methane-oxidizing bacteria (methanotrophs) are ubiquitous, acting as filters for methane in the environments and play a central role in processes affecting climate change. Even though methanotrophs were intensively studied in the last decades, little is known about the feasibility of comparing the experimental results across the different laboratories performing the same procedures. Similarly, little is known about how methanotroph function and community composition are influenced by the overall microbial diversities; their resistance and resilience against disturbances regarding their community structure, abundance, and function.

10.4.1 Methanotrophs

The methanotrophs are a subgroup of the methylotrophs and are generally characterized by their ability to use methane as their sole carbon and energy source (Hanson and Hanson 1996). The key methanotrophic enzyme is methane monooxygenase (MMO), which occurs as both particulate (pMMO) and soluble (sMMO) forms. The *pmoA* gene encodes the α-subunit of pMMO and is included in the genome of majority of known methanotrophs except *Methylocella* and *Methyloferula* (Dedysh et al. 2000; Vorobev et al. 2011).

Methanotrophic bacteria are found in three phyla—*Proteobacteria*, *Verrucomicrobia*, and NC10. The traditional methanotrophs belong to the phylum Proteobacteria. Recently, methanotrophs in the phylum *Verrucomicrobia* have been discovered but they seem to be restricted to extreme environments (Dunfield et al. 2007; Pol et al. 2007; Islam et al. 2008). The novel phylum, NC10, represents bacteria capable of aerobic methane oxidation coupled to denitrification under anoxic conditions (Ettwig et al. 2010). Moreover, ammonia oxidizers were also shown to be able to convert methane to methanol by an enzyme homologous to the methane monooxygenase of methanotrophs. It seems, however, that they cannot grow using this process (Hyman and Wood 1983; Jones and Morita 1983).

Proteobacterial methanotrophs could be further divided into type I (γ-*Proteobacteria*, families *Methylococcaeae* and *Methylothermaceae*) and type II (α-*Proteobacteria*, families *Methylocystaceae* and *Beijerinkiaceae*) according to their morphologies, physiologies, and phylogenies (Trotsenko and Murrell 2008).

The phylum *Verrucomicrobia* methanotrophs were found to grow at low pH around 1–2 and high temperatures over 55 °C. Complete genome sequencing of one isolate suggested that the genes essential for methanotrophy came from the *Proteobacteria* by horizontal gene transfer (Hou et al. 2008). In addition, besides of *Proteobacteria* and *Verrucomicrobia*, there are a number of environmental sequences retrieved from culture-independent studies.

Methanotrophs are divided into at least two functionally distinct groups, the high-affinity group that uses CH_4 at very low concentrations, and the low-affinity group that only uses CH_4 at high concentrations (Bender and Conrad 1992). Most of the culturable methanotrophs are low affinity, which tend to be located near source environments (Reaya et al. 2005).

In addition to the more common methanotrophs, a group of methanogen-like anaerobic CH_4-oxidizing archaea (MOA) has been described (Hallam et al. 2003). These MOA contain *mcrA* genes and many are involved in a consortium that couples denitrification with anaerobic CH_4 oxidation (Raghoebarsing et al. 2006).

Until 2005, methanotrophs were regarded as obligatory utilizing one-carbon compounds for growth, but then it was reported that *Methylocella* could utilize multi-carbon compounds besides methane (Dedysh et al. 2005). *Crenothrix polyspora* a sheathed γ-Proteobacteria was identified to be another possible candidate for a facultative methanotroph (Stoecker et al. 2006). More recently, pMMO-possessing methanotroph of the genus *Methylocapsa*, as well as some *Methylocystis* species, was demonstrated to be able to grow on acetate as a sole substrate (Belova et al. 2011; Dunfield et al. 2010). The facultative lifestyle in methanotrophs indicates that broader substrate utilization might be more common in methanotrophs as previously thought.

The capacity to produce or consume CH_4 is distributed among relatively few microbial taxa that are phylogenetically distinct (Martiny et al. 2013), and this imply that CH_4 production and consumption rates may be more closely tied to microbial community composition and abundance than other biogeochemical processes (Schimel 1995). Genes involved in methane cycling are found in deep-branching microbial clades, similar to other complex microbial traits such as oxygenic photosynthesis and sulfate reduction (Martiny et al. 2013). By contrast, genes involved in the heterotrophic processing of other carbon compounds are not highly conserved, and nearly all microbial taxa contribute to CO_2 production in upland soils.

10.4.2 Cultivation-Independent Approaches in Study of Soil Methanotrophs

Upland and forest soils are regarded as major sinks for atmospheric methane, and high-affinity methanotrophs are proposed to be responsible for this process. But up to now, putative methanotrophs have only been defined by the environmental clones grouped as the upland soil cluster (USC), e.g., USC-γ and USC-α (Knief et al. 2003; Knief and Dunfield 2005).

Due to the difficulties in cultivating methanotrophs, various efforts have been undertaken to explore the methanotrophic diversity by cultivation-independent approaches**.** Cultivation recovers only a small fraction of the cells present in the environmental sample and does not allow the so-called uncultured forms to be analyzed A variety of methods exist to characterize the microbial community at different resolution levels, and each of them has benefits and constraints. For example, widely used PCR-based DNA fingerprinting and quantification methods are able to provide high-resolution taxonomic information, are reproducible and suitable for high-throughput analysis. However, DNA-based techniques have methodological bias during nucleic acid extraction from soil and amplification and are unable to differentiate active, dormant, or dead sources of DNA.

Different methods are currently applied to study methanotrophs diversity in soils, including immunofluorescence analysis (IFA), fluorescent in situ hybridization (FISH), phospholipids fatty acids (PLFA) analysis, and PCR amplification of using specific primers coupled to denaturating gradient gel electrophoresis (DGGE) or cloning. Stable isotope probing (SIP), an attractive method to link bacteria to their functions, has been also used extensively, in relation to aerobic methane oxidation**.** Holmes et al. (1999) revealed an unknown group of methanotrophic bacteria, exhibiting similarities to type II methanotrophs, using ^{14}C-phospholipid ester-linked fatty acid profiles. Type I and II, high affinity, uncultured methanotrophs were observed in upland soils using fatty acid methyl ester analysis (FAME) (Seghers et al. 2003) and PCR-DGGE analysis (Fjellbirkeland et al. 2001). Direct microscopic techniques are widely applied for in situ identification of methanotrophs. However, FISH is targeting active growing bacterial cells and fails to reveal resting microorganisms. IFA permits detection of both active and resting cells, but this method is time-consuming and restricted to a determined suit of microorganisms as compared to molecular techniques.

10.4.3 Labelled-Antibody Microscopy Assay

Serological methods for the detection of target microorganisms are based on a reaction of antigenic determinants with antibodies. Polyclonal antisera, produced after immunization of rabbits with pure cultures of methanotrophs, may be used for specific detection of target bacteria. For soil, IFA method is the most reliable and sensitive detection of target bacteria in soil. The sensitivity of the method is about 10^3–10^4 cell mL^{-1} (Galchenko et al. 1988), and it can be applied for detection and quantification of target bacteria. The technique for identification and enumeration of selected methanotrophic species in environmental samples using the indirect immune fluorescent antibodies was developed and applied for the analysis of tundra soils (Vecherskaya et al. 1993) and upland soils of Russia (Kravchenko et al. 2005) and Belgium (Bykova et al. 2007). It was shown that results of IF analysis of the mixed methanotrophic cultures were in good agreement with cultural and molecular data (Slobodova et al. 2006). The limited number of fluorescent species-specific antibodies is the main weakness of IFA in the analysis of the structure of methanotrophic communities.

10.4.4 FISH Analysis

The fluorescence in situ hybridization (FISH) method, which combines identification and enumeration of MOB, is based on the detection of rRNA fragments and depends on the physiological state of microorganisms (Bouvier and del Giorgio 2003). FISH targeting the 16S rRNA gene has been used to identify (Eller et al. 2001) and enumerate (Dedysh et al. 2003) methanotrophs in different environments, including forest soils (Lau et al. 2007). The disadvantages of using FISH are that it can only be used when the population is numerous enough to be counted under the microscope and when the 16S rRNA genes of the target organisms are known. Due to the many diversity studies of methanotrophs using *pmoA* phylogeny, many novel groups of methanotrophs can only be identified by *pmoA* sequence; hence, FISH cannot be used to enumerate these organisms.

10.4.5 PLFA Analysis

The investigation of specific phospholipid acids provides reliable estimates of the biomass and cell numbers of MOB but does not reveal the taxonomic structure of the community (Sundh et al. 1995a, b). Type I methanotrophs produce C_{16} fatty acids as their most abundant PLFAs, whereas type II methanotrophs produce a higher concentration of C_{18} fatty acids (Hanson and Hanson 1996). However, a recent study showed that *Methylocystis heyeri* strains (type II methanotrophs) contained large amounts of 16:1ω8c, a phospholipid fatty acids (PLFA) that was previously thought to be associated with type I methanotrophs only (Dedysh et al. 2007). The major disadvantage of PLFA analysis is that it is not precise enough to identify bacteria to the species level. The specificity of PLFA profiling of bacterial populations can be significantly enhanced by applying isotopically labelled substrates to soils or sediments.

10.4.6 Stable Isotope Probing

Stable isotope probing (SIP) is a method to identify the active microorganisms responsible for selected environmental functions in situ. In SIP, growth substrates labelled with stable isotopes such as ^{13}C, ^{15}N are added to environmental samples, resulting in these elements being used as carbon or nitrogen source and incorporated into DNA (Radajewski et al. 2000), RNA (Manefield et al. 2002), PLFA (Boschker and Middelburg 2002), or proteins (Jehmlich et al. 2010).

DNA, RNA, and PFLA-SIP have been applied in methanotroph diversity studies. DNA/RNA or PFLA-SIP have been extensively used by Murrell and his coworkers to discover the methanotrophic bacteria in different habitats, e.g., the Movile Cave (Hutchens et al. 2004), Transbaikal soda lake sediments (Lin et al. 2004), landfill cover soil (Cébron et al. 2007), acidic peatlands (Chen et al. 2008), alkaline soil (Han 2009), and pine forest soil (Bengtson et al. 2009).

One of the key limitations of DNA-SIP are the long incubation times and "cross-feeding" problems. Recently, this method has been improved by shorter incubation times and lower substrate concentrations (Chen et al. 2008).

10.4.7 Diagnostic Microarray Analysis

Microarrays consist of an orderly arrangement of probes (oligonucleotides, DNA fragments, proteins, sugars) attached to a solid surface. To date, microbial diagnostic microarrays (MDM) have been developed and widely applied to the microbial ecology study as well. They contain oligonucleotide probes, which are specific for a given strain, subspecies, species, genus, or higher taxon (Bodrossy et al. 2003). The main advantages of MDM are parallelism, short time, high reproducibility, and resolution. In methanotroph studies, pmoA-based functional MDMs have been successfully employed. This *pmoA*-based microarray (Bodrossy et al. 2003) employs short oligonucleotides (18–27 nucleotides) as probes against the *pmoA* genes of MOB, including environmental clones. Fluorescently labelled nucleic acids of unknown samples (targets) are hybridized to the probes and analysis can be performed with DNA and mRNA. The *pmoA*-array has been widely applied to study methanotroph diversity (Abel et al. 2009; Bodelier et al. 2009; Gebert et al. 2009).

10.4.8 PCR-Based Analysis of Methanotrophs Diversity

Yet uncultured methanotrophs can be detected with nucleic acid probes or by sequencing genes amplified by PCR directly from environmental samples. These methods are useful for identification of taxa and for determination of the phylogenetic positions of microbes. Recently, the application of molecular methods for soil microbiology were comprehensively reviewed (Kirka et al. 2004; Leckie 2005; Malik et al. 2008; Sharma et al. 2007; Smith and Osborn 2009; Haruta 2013; Lynch and Neufeld 2015).

Large numbers of 16S rRNA gene probes have been designed to amplify methanotrophs and to date, quite a few of these sets of methanotroph-specific 16S rRNA probes have been used in environmental studies. Study of functional genes is valuable because it leads to a better understanding of the activity of bacteria in different environments and their role in the methane cycling. An advantage of using functional genes instead of 16S rRNA to study bacterial diversity is that they enable the potential functional diversity within an environment to be investigated. The methods involving the analysis of *pmoA* and *mmoX*, the functional marker genes of methanotrophy, make it possible to detect both known and novel methanotrophs, but are not universal because *Methylocella and Methyloferula* lack the *pmoA* gene and only a few methanotrophs have the *mmoX* gene. Sequence analysis of *nifH,* the marker gene of nitrogen fixation, was recently demonstrated to be applicable for the successful identification of methanotrophic bacteria (Boulygina et al. 2002; Dedysh et al. 2004).

The first oligonucleotide primers designed to amplify internal fragments of the genes encoding pMMO and AMO (ammonia monooxygenase) enzyme complexes were the A189f/A682r (Holmes et al. 1999) The phylogeny of *pmoA/amoA* is reasonably congruent with the 16SrRNA gene phylogeny of the organisms from which the gene sequences were retrieved (Holmes et al. 1999; Kolb et al. 2003); therefore, *pmoA* and *amoA* sequences provide information on the phylogenetic position of these organisms. Different *pmoA* primer combinations target different groups of MOB (Holmes et al. 1995; Costello and Lindstrom 1999; Bourne et al. 2001). The A189f/A682r primers have been used extensively in environmental studies to provide a molecular profile and the diversity of the methanotrophs and the related *amoA* gene in various environments (Bourne et al. 2001; Holmes et al. 1999; Horz et al. 2002, 2001; Kolb et al. 2003) and have proved useful in detecting novel sequences (Holmes et al. 1999; Knief et al. 2003). A new reverse *pmoA*-specific primer mb661r was designed and demonstrated specificity in amplifying *pmoA*, but not *amoA,* but does not address the high-affinity methanotrophs, which can be detected by other primer systems (Bourne et al. 2001; Shrestha et al. 2008). Another potentially useful marker is the *mxaF* gene which was used for the study of methanotrophs diversity in marine, soil, and wetland samples (Holmes et al. 1995; McDonald et al. 2008). However, this gene is not specific for methanotrophs and also occurs in methylotrophs unable to use CH_4.

PCR primer sequence development and protocol selection also affect the accuracy of amplification-based community analysis techniques. Primers for functional genes are unlikely to capture the full diversity of the target genes for which they are designed, due to the high divergence of nucleotide sequences at current primer sites (Green et al. 2010). Another limitation is the absence of a complete database of functional gene sequences. Targeted functional gene studies and metagenomic surveys can be used to extend gene sequence and metagenomic datasets for functional genes, which can identify novel sequences of known functional genes (Myrold et al. 2013). Design and application of the new more comprehensive primers for functional gene analysis may provide an accurate estimation of the linkages between functional genes and environmental processes.

The presence of functional genes does not always indicate an active community. An alternative method of linking functional communities in the soil to process rates is qPCR of mRNA transcripts of functional genes, which provide an estimate of gene expression from metabolically active microbial cells. Studies examining functional gene transcript abundance and attempting to link this measure to process rates have largely taken place in laboratory incubations or microcosms (Nicolaisen et al. 2008; Freitag and Prosser 2009; Liu et al. 2010). In situ field estimates of functional gene activity are less common, but have provided important links between functional gene activity and process rates. For example, *pmoA* gene: transcript ratio was negatively correlated with CH_4 flux rates at a different peat bog site (Freitag et al. 2010). However, researchers have struggled to detect functional gene transcriptional activity under field conditions, including denitrification genes (Liu et al. 2010).

The 16S rRNA gene is the most frequently used phylogenetic marker to determine microbial evolutionary relationships and for microbial diversity studies.

Due to the large database and its conservative nature, the 16S rRNA gene is also widely used in the analysis of methanotrophs. However, it is rather difficult to design specific 16S rRNA primers. Recently, Chen et al. (2008) designed 16S rRNA gene primer sets targeting type I and type II methanotrophs, however, specificity is not warranted.

10.4.8.1 Denaturing Gradient Gel Electrophoresis (DGGE)

Both 16S rRNA genes and *pmoA* were applied as targets in DGGE for analyzing methanotroph communities. This method is based on the electrophoretic separation of DNA fragments, which have the same length but different sequences. The PCR-DGGE fingerprinting was successfully applied in ecology studies of soil methanotrophs (Kravchenko et al. 2010). However, the use of degenerate primers, which are sometimes needed to cover methanotroph diversity, often generates multiple bands for a single organism. Recently, new 16S rDNA primer sets were designed (Chen et al. 2008) which could cover almost all known methanotrophs excluding *Verrucomicrobia* and applied to study MOB diversity in landfill soils. New *pmoA*-based nondegenerate primers (mb661_nd) were designed in order to avoid multiple band production and were successfully used for the detection MOB in an alkaline Mono lake (Lin et al. 2005).

10.4.8.2 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP is an alternative method to fingerprint methanotroph communities and is often regarded to be rapid, sensitive, semiquantitative, and highly reproducible. Since Horz et al. (2001) first applied *pmoA*-based T-RFLP analysis to study the diversity of methanotrophs on rice roots; there have been a number of studies based on this method (Gebert et al. 2009; Lüke et al. 2010).

10.4.8.3 Sequence Analysis

Cloning and Sanger sequencing technology are widely used in microbial ecology studies. Methanotroph community diversity and single methanotroph genome analysis were largely investigated by Sanger sequencing. Next-generation high-throughput sequencing (HTS) such as 454 pyrosequencing and Illumina sequencing technologies have recently revolutionized microbial community analysis. HTS allows generating massive sequence data in order to get sufficient depth to resolve biological patterns. This technology has been successfully applied for studying microbial 16S rRNA gene diversity in a number of environments (Roesch et al. 2007; Degnan and Ochman 2012; Liu et al. 2015), as well as for methanotroph communities composition analysis. The 16S rRNA gene as pyrosequencing tag has been used for studying anaerobic methane oxidizers (ANME) communities in cold seep

sediments (Roalkvam et al. 2011). The *pmoA* gene has been usually chosen as amplicon pyrosequencing tag for aerobic methanotroph communities studies (Kip et al. 2011; Lüke and Frenzel 2011).

10.4.9 Quantification of Methanotrophs

The most probable number (MPN) technique has been widely used in the past for quantifying methanotrophs in the environment. The method is limited by the fact that only a fraction of microorganisms can be cultivated. Quantitative, real-time PCR is widely used to quantify microbes from environmental samples. Quantitative PCR (qPCR) uses fluorescent dyes (e.g., SYBR green) or oligonucleotide probes (e.g., Taq man probes). A quantitative real-time PCR assay for different types of methanotrophs using SYBR green and *pmoA*-specific primers were developed (Kolb et al. 2003). This assay has been subsequently used to quantify the methanotrophs; however, it has been suggested that this assay might underestimate methanotroph populations. Real-time PCR targeting 16S rRNA genes has been applied for methanotroph quantification as well (Halet et al. 2006); however, the primers used in this study were not specific for methanotrophs and thus likely overestimated MOB populations. The quantitative real-time PCR assay for methanotrophs was developed from a method using SYBR green and pmoA-specific primers designed to target five different groups of methanotrophs in real-time PCR (Kolb et al. 2003). This assay was successfully used to quantify the methanotroph community in a number of environments, including forest soils (Kolb et al. 2005).

Through the use of barcoded PCR primers targeting the V3 region of microbial 16S rRNA gene, the Illumina platform has high depth coverage of microbial diversity and allows the assessment of microbiota both qualitatively (to determine diversity) and quantitatively. To the best of our knowledge, HTS technique has not been used to compare the diversity and abundances between groups of methanotrophic bacterial communities (e.g., members of the *Methylococcaceae* versus members of the *Methylocystaceae*) within one habitat and between neighboring habitats within forest soil ecosystems. Knowledge from comparisons of methanotroph community structures based on 16S rRNA diversity patterns may identify habitat-specific adaptations in methanotroph physiology and evolution.

10.4.10 Methanotrophic Communities of Native and Agricultural Soils of European Russia

Soils consuming atmospheric CH_4 are significant global sinks of methane, where CH_4 is consumed by aerobic methanotrophic bacteria. However, to the best of our knowledge, no study has compared methanotroph diversity and abundances between these different environments with high methane oxidation rates, which are in close proximity to each other.

The IFA analysis of native forest and agrocenosis *gray forest* soil revealed principal differences in aerobic methanotrophs communities (Kravchenko et al. 2005). The dominance of *Methylocystis* (about 94 % of identified methanotrophs number) was found in native soil, but in the arable soil it was a minor component of the community (less than 6 %). By contrast, the number of *Methylobacter* in the forest soil was under the detection threshold, whereas, in the arable soil, this species dominated and accounted for 57.1 % of the net population of methanotrophs. The total number of IFA identified methanotrophs in samples of forest soil was roughly ten times higher than that in arable soil, but the community structure was homogeneous, being represented only *Methylocystis.* Conversely, the methanotrophic community of agricultural soil consisted of *Methylocystis, Methylomonas,* and *Methylobacter* (Kravchenko et al. 2005). These data are consistent with results of *pmoA* cloning and FISH analysis in forest soil, which show the *Methylococcaceae* to be the minor component of the methanotrophic populations (Lau et al. 2007, 2015). Alternately, in agricultural Belgian soils with high atmospheric methane oxidation *Methylocystis* and *Methylosinus* outnumbered type I methanotrophs (Bykova et al. 2007).

The oxidation of atmospheric methane in the forest, arable, and other aerobic soils was earlier proven convincingly to be connected with the activity of methanotrophic bacteria, but it is not known which methanotrophs carry out this process. Based on studies of methane oxidation kinetics, as far as studies of microbial communities of aerobic soils carried out with the use of *pmoA* gene analysis (Dunfield et al. 1999) and radioactive fingerprinting (Roslev Iversen 1999), it was suggested the existence of high-affinity methanotrophs (nanomolar values of K_m) responsible for atmospheric methane oxidation. An enrichment soil methanotrophic culture was experimentally adapted to nanomolar values of K_m close to those found in aerobic soils (Dunfield et al. 1999). *Methylocystis* and *Methylosinus* containing enrichments from the forest and agricultural soils were shown to oxidize methane at atmospheric concentrations (k_m 54.2–176.8 nM CH_4), probably due to the presence of the *pmoA*2 gene (Kravchenko et al. 2010).

Study of aerobic methanotrophs diversity in eight most typical soil types (both unmanaged and agricultural) distributed across the European part of Russia are in good agreement with data for *gray forest* soil (Kizilova et al. 2013). The analysis of *pmoA* clone libraries demonstrated less diversity of the methanotrophic populations in unmanaged soils than in agricultural areas. These clone sequences formed three groups of uncultured methanotrophs: USC-gamma, cluster I, and *pmoA*/*amoA* cluster, which are believed to be responsible for atmospheric methane oxidation in upland soils. Agricultural soils harbored methanotrophs related to *Methylosinus, Methylocystis, Methylomicrobium, Methylobacter,* and *Methylocaldum.* Despite higher numbers of detected molecular operational taxonomic units (MOTUs), managed soils showed decreased methane oxidation rates as observed in both in situ and laboratory experiments.

Our study demonstrated that uncultured NSUC (natural soil uncultivated cluster) methanotrophs with *pmoA/amoA* monooxygenase dominated in methane-oxidizing communities in unmanaged soils of forest and steppe zone of Russia

(Kravchenko et al. 2014). Further studies were addressed to the studying of this novel group in sod-podzolic soil (Haplic Albeluvisol in FAO classification) of Moscow Region. PCR-DGEE analysis of *pmoA* revealed the predominance of uncultured methanotrophs in forest soil and high diversity of type I and type II methanotrophs in agrosoil. Analysis of *pmoA* clone libraries has demonstrated that methanotrophs of the forest side are the part of the compact *pmoA*/*amoA* cluster of formed by environmental clones from different unmanaged soils. A new *pmoA*-specific primer set was designed, and the number uncultured methanotrophs was $(9.2 \pm 0.87) \times 10^4$ copies g^{-1}, and transcripts number $(1.33 \pm 0.31) \times 10^6$ g^{-1}.

10.4.11 The Link to Methanotrophs Functional Rates and Diversity

Some studies have revealed close relationships between methane oxidation rates and community structure, often in the context of environmental change. In a temperate agricultural soil, long-term N-fertilization resulted in simultaneously reduced methanotroph abundance decline in methane oxidation rates (Seghers et al. 2003; Maxfield et al. 2008). Different groups of methanotrophs may show different responses to fertilization as observed in forest soils where type II methanotrophs were more strongly inhibited by mineral N fertilization than type I methanotrophs (Mohanty et al. 2006). In contrast, organic fertilizer addition can increase methanotroph abundance and associated rates of methane oxidation in agricultural soils (Seghers et al. 2006; Bykova et al. 2007; Ho et al. 2015). In pine forest soil, methane oxidation rates across soil horizons are correlated with the abundance of methanotrophs PLFA marker (Bengtson et al. 2009). The link was found between methane consumption and type I methanotrophs abundance at a riparian floodplain (Bodelier et al. 2013) and Mediterranean woodlands (Shvaleva et al. 2015) but in studies of afforestation and reforestation the link of type II methanotrophs abundance and higher CH_4 uptake was recorded (Singh et al. 2007; Nazaries et al. 2011). The pronounced shift in methanotroph diversity, as far as abundance were found to be directly linked with high-affinity methane oxidation in Russian agricultural soils (Kravchenko et al. 2005; Kizilova et al. 2013).

Sometimes no relationship between methanotrophs abundance or diversity and methane uptake was found. *PmoA* genes associated with high-affinity methanotrophs were found in glacial field in Greenland, but CH_4 oxidation was not detected (Bárcena et al. 2011). Differences in methanotroph community composition but not in methane oxidation were found in response to chronic herbicide treatment (Seghers et al. 2003).

It may be suggested that environment can select for or influence methanotroph community structure and may affect methanotroph biogeography as previously suggested for paddy soil methanotrophs (Lüke et al. 2010).

10.5 Conclusions

Methane-oxidizing soil microorganisms have the great potential to impact the atmospheric composition of the Earth. Russia is extremely rich in soil types due to its vast territories, and most of these soils have never been investigated from the aspect of methanotrophy. We hypothesized that net CH_4 flux would be correlated with the activity, abundance, and/or composition of methane-cycling microbes. In fact, we have found satisfactory evidence that the impacts of environmental drivers, as far as anthropogenic disturbance on net CH_4 flux are the result of changes in the methane-cycling microbial community. The environmental factors are the controllers of the methanotrophic activity, while anthropogenic transformation resulted in significant changes in methanotrophic diversity and abundance. Methanotrophic population of unmanaged soils turned out to be much low diverse and dominated by uncultivated methanotrophs. Pronounced shift to larger diversity with high similarity with the cultured methanotrophs was evaluated in the same soils after agricultural loading, but they failed to make a significant contribution to elimination of methane. The studies reviewed here provide a framework for the use of microbial functional gene analysis I to fill gaps in our knowledge of non-wetland terrestrial ecosystems and farming systems functions such as methane-cycling processes.

Acknowledgements Financial support from Russian Foundation for Basic Research (grants 13-04-00603 and 16-04-00136) is gratefully acknowledged.

References

Abel GCJ, Stralis-Pavese N, Sessitsch A, Bodrossy L (2009) Grazing affects methanotroph activity and diversity in an alpine meadow soil. Environ Microbiol Rep 1:457–465

Acton SD, Baggs EM (2011) Interactions between N application rate, CH_4 oxidation and N_2O production in soil. Biogeochemistry 103:15–26

Adamsen APS, King GM (1993) Methane consumption in temperate and subarctic forest soils: rates, vertical zonation, and responses to water and nitrogen. Appl Environ Microbiol 59:485–490

Aronson EL, Allison SD, Helike BR (2013) Environmental impacts on the diversity of methane cycling microbes and resultant functions. Front Microbiol 4:47–56

Bárcena TG, Finster KW, Yde JC (2011) Spatial patterns of soil development, methane oxidation, and methanotrophic diversity along a receding glacier forefield, Southeast Greenland. Arct Antarct Alp Res 43:178–188

Belova SE, Baani M, Suzina NE, Bodelier PLE, Liesack W, Dedysh SN (2011) Acetate utilization as a survival strategy of peat-inhabiting *Methylocystis* spp. Environ Microbiol Rep 3:36–46

Bender M, Conrad R (1992) Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH_4 mixing ratios. FEMS Microbiol Ecol 101:261–270

Bender M, Conrad R (1993) Kinetics of CH_4 oxidation in oxic soils. Chemosphere 26:687–696

Bengtson P, Basiliko N, Dumont MG, Hills M, Murrell JC, Roy R, Grayston SJ (2009) Links between methanotroph community composition and CH_4 oxidation in a pine forest soil. FEMS Microbiol Ecol 70:356–366

Billings SA, Richter DD, Yarie J (2000) Sensitivity of soil methane fluxes to reduced precipitation in boreal forest soils. Soil Biol Biochem 32:1431–1441

Bodelier PLE (2011) Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. Curr Opin Environ Sustain 3:379–388

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiol Ecol 47:265–277

Bodelier PLE, Kamst M, Meima-Franke M, Stralis-Pavese N, Bodrossy L (2009) Whole-community genome amplification (WCGA) leads to compositional bias in methane-oxidizing communities as assessed by *pmoA*-based microarray analyses and QPCR. Environ Microbiol Rep 1(5):434–441

Bodelier PLE, Meima-Franke M, Hordijk CA, Steenbergh AK, Hefting MM, Bodrossy L, von Bergen M, Seifert J (2013) Microbial minorities modulate methane consumption through niche partitioning. ISME J 7:2214–2228

Bodrossy L, Stralis-Pavese N, Murrell JC, Radajewski S, Weilharter A, Sessitsch A (2003) Development and validation of a diagnostic microbial microarray for methanotrophs. Environ Microbiol 5:566–582

Boeckx P, Van Cleemput O (1996) Methane oxidation in a neutral landfill cover soil: influence of moisture content, temperature, and nitrogen-turnover. J Environ Qual 25:178–183

Boeckx P, Van Cleemput O, Villarvo I (1997) Methane oxidation in soils with different textures and land use. Nutr Cycl Agroecosyst 49:91–95

Börjesson G, Chanton J, Svensson BH (2001) Methane oxidation in two Swedish landfill covers measured with carbon-13 to carbon-12 isotope ratios. J Environ Qual 30:369–376

Borken W, Davidson EA, Savage K, Gaudinski J, Trumbore SE (2003) Drying and wetting effects on carbon dioxide release from organic horizons. Soil Sci Soci Am J 67:1888–1896

Born M, Dorr H, Levin N (1990) Methane consumption in aerated soils of the temperate zone. Tellus B 42:2–8

Boschker HTS, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. FEMS Microb Ecol 40:85–95

Boulygina ES, Kuznetsov BB, Marusina AI, Tourova TP, Kravchenko IK, Bykova SA, Kolganova TV, Gal'chenko VF (2002) A study of nucleotide sequences of *nifH g*enes of some methanotrophic bacteria. Microbiology 71:425–432

Bourne DG, McDonald IR, Murrell JC (2001) Comparison of *pmoA* PCR primer sets as tools for investigating methanotroph diversity in three Danish soils. Appl Environ Microbiol 67:3802–3809

Bousquet P, Ciais P, Miller JB, Dlugokencky EJ, Hauglustaine DA, Prigent C, Van der Werf GR, Peylin P, Brunke EG, Carouge C, Langenfelds RL, Lathière J, Papa F, Ramonet M, Schmidt M, Steele LP, Tyler SC, Whitel J (2006) Contribution of anthropogenic and natural sources to atmospheric methane variability. Nature 443:439–443

Bouvier T, del Giorgio PA (2003) Factors influencing the detection of bacterial cells using fluorescence in situ hybridization (FISH): a quantitative review of published reports. FEMS Microbiol Ecol 44:3–15

Bowden RD, Newkirk KM, Rullo GM (1998) Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. Soil Biol Biochem 30:1591–1597

Bykova S, Boeckx P, Kravchenko I, Galchenko V, Van Cleemput O (2007) Impact of fertilization type and CH_4 concentration on methane oxidation and methanotrophic diversity in arable soils. Biol Fertil Soils 43:341–348

Castro MS, Steudler PA, Melillo JM, Aber JD, Bowden RD (1995) Factors controlling atmospheric methane consumption by temperate forest soils. Glob Biogeochem Cycles 9:1–10

Castro MS, Gholz HL, Clark KL, Steudler PA (2000) Effects of forest harvesting on soil methane fluxes in Florida slash pine plantations. Can J For Res 30:1534–1542

Cébron A, Bodrossy L, Chen Y, Singer AC, Thompson IP, Prosser JA, JC M (2007) Identity of active methanotrophs in landfill cover soil as revealed by DNA-stable isotope probing. FEMS Microb Ecol 62:12–23

Chan ASK, Parkin TB (2001) Methane oxidation and production activity in soils from natural and agricultural ecosystems. J Environ Qual 30:1896–1903

Chen Y, Dumont MG, McNamara NP, Chamberlain PM, Bodrossy L, Stralis-Pavese N, Murrell JC (2008) Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PLFA analyses. Environ Microbiol 10:446–459

Conrad R, Rothfuss F (1991) Methane oxidation in the soil surface layer of a flooded rice field and the effect of ammonium. Biol Fertil Soils 12:28–32

Costello AM, Lindstrom ME (1999) Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Appl Environ Microbiol 65:5066–5074

De Visscher A, Schippers M, Van Cleemput O (2001) Short-term kinetic response of enhanced methane oxidation in landfill cover soils to environmental factors. Biol Fertil Soils 33:231–237

Dedysh SN, Belova SE, Bodelier PL, Smirnova KV, Khmelenina VN, Chidthaisong A, Trotsenko YA, Liesack W, Dunfield PF (2007) Methylocystis heyeri sp. nov., a novel type II methanotrophic bacterium possessing 'signature' fatty acids of type I methanotrophs. Int J Syst Evol Microbiol 57:472–479

Dedysh SN, Liesack W, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Bares AM, Panikov NS, Tiedje JM (2000) *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. Int J Syst Evol Microbiol 3:955–969

Dedysh SN, Dunfield PF, Derakshani M, Stubner S, Heyer J, Liesack W (2003) Differential detection of type II methanotrophic bacteria in acidic peatlands using newly developed 16S rRNA-targeted fluorescent oligonucleotide probes. FEMS Microbiol Ecol 43:299–308

Dedysh SN, Ricke P, Liesack W (2004) NifH and NifD phylogenies: an evolutionary basis for understanding nitrogen fixation capabilities of methanotrophic bacteria. Microbiology 150:1301–1313

Dedysh SN, Knief C, Dunfield PF (2005) *Methylocella* species are facultatively methanotrophic. J Bacteriol 187(13):4665–4670

Degelmann DM, Borken W, Drake HL, Kolb S (2010) Different atmospheric methane-oxidizing communities in European beech and Norway spruce soils. Appl Environ Microbiol 76:3228–3235

Degnan PH, Ochman H (2012) Illumina-based analysis of microbial community diversity. ISME J 6:183–194

Dorr N, Glaser B, Kolb S (2010) Methanotrophic communities in Brazilian ferralsols from naturally forested, afforested, and agricultural sites. Appl Environ Microbiol 76:307–1310

Dunfield PF (2007) The soil methane sink. In: Reay D, Hewitt CN, Smith K, Grace J (eds) Greenhouse gas sinks. CAB International, Wallingford, pp 152–170

Dunfield PF, Knowles R (1995) Kinetics of inhibition of methane oxidation by nitrate, nitrite, and ammonium in a humisol. Appl Environ Microbiol 61:3129–3135

Dunfield PF, Liesack W, Henckel T, Knowles R, Conrad R (1999) High-affinity methane oxidation by a soil enrichment culture containing a Type II methanotroph. Appl Environ Microbiol 65:1009–1014

Dunfield PF, Belova SE, Vorob'ev AV, Cornish SL, Dedysh SN (2010) *Methylocapsa aurea* sp. nov., a facultatively methanotrophic bacterium possessing a particulate methane monooxygenase. Int J Syst Evol Microbiol 60:2659–2664

Dutaur L, Verchot LV (2007) A global inventory of the soil CH_4 sink. Glob Biogeochem Cycles 21:1–9

Eller G, Stubner S, Frenzel P (2001) Group-specific 16S rRNA targeted probes for the detection of Type I and Type II methanotrophs by fluorescence in situ hybridization. FEMS Microbiol Lett 198:31–37

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MM, Schreiber F, Dutilh BE, Zedelius J, de Beer D, Gloerich J, Wessels HJ, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HJ, Janssen-Megens EM, Francoijs KJ, Stunnenberg H,

Weissenbach J, Jetten MS, Strous M (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464:543–548

Fjellbirkeland A, Torsvik V, Ovreas L (2001) Methanotrophic diversity in an agricultural soil as evaluated by denaturing gradient gel electrophoresis profiles of *pmoA*, *mxaF* and 16S rDNA sequences. Antonie Van Leeuwenhoek 79:209–217

Flechard CR, Neftel A, Jocher M, Ammann C, Fuhrer J (2005) Bi-directional soil/atmosphere N_2O exchange over two mown grassland systems with contrasting management practices. Global Change Biol 11:2114–2127

Freitag TE, Prosser JI (2009) Correlation of methane production and functional gene transcriptional activity in a peat soil. Appl Environ Microbiol 75:6679–6687

Freitag TE, Toet S, Ineson P, Prosser JI (2010) Links between methane £ux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. FEMS Microb Ecol 73:157–165

Galchenko VF, Abramochkina FN, Bezrukova LV, Sokolova EN, Ivanov MV (1988) The species structure of aerobic methanotrophic microflora in the Black Sea. Microbiology 57:305–311

Gebert J, Singh BK, Pan Y, Bodrossy L (2009) Activity and structure of methanotrophic communities in landfill cover soils. Environ Microbiol Rep 1:414–423

Goldman MB, Groffman PM, Pouyat RV, McDonnell MJ, Pickett STA (1995) CH_4 uptake and N availability in forest soils along an urban to rural gradient. Soil Biol Biochem 27:281–286

Green SJ, Prakash O, Gihring TM, Akob DM, Jasrotia P, Jardine PM, Watson DB, Brown SD, Palumbo AV, Joel E, Kostka JE (2010) Denitrifying bacteria from the terrestrial subsurface exposed to mixed waste contamination. Appl Environ Microbiol 76:3244–3254

Gulledge J, Schimel JP (1998) Low-concentration kinetics of atmospheric CH_4 oxidation in soil and mechanism of NH_4^+ inhibition. Appl Environ Microbiol 64:4291–4298

Halet D, Boon N, Verstraete W (2006) Community dynamics of methanotrophic bacteria during composting of organic matter. J Biosci Bioeng 101:297–302

Hallam SJ, Girguis PR, Preston CM, Richardson PM, DeLong EF (2003) Identification of methyl coenzyme M reductase A (*mcrA*) genes associated with methane-oxidizing *Archaea*. Appl Environ Microbiol 69:5483–5491

Han B, Chen Y, Abell G, Jiang H, Bodrossy L, Zhao J, Murrell JC, Xing X-H (2009) Diversity and activity of methanotrophs in alkaline soil from a Chinese coal mine. FEMS Microbiol Ecol 70:196–207

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471

Haruta S (2013) Rediscovery of the microbial world in microbial ecology. Microbes Environ 28:281–284

Ho A, Reim A, Kim SY, Meima-Franke M, Termorshuizen A, de Boer W, van der Putten WH, Bodelier PL (2015) Unexpected stimulation of soil methane uptake as emergent property of agricultural soils following bio-based residue application. Global Change Biol 10:3864–3879

Holmes AJ, Costello A, Lidstrom ME, Murrell G (1995) Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. FEMS Microbiol Lett 132:203–208

Holmes AJ, Roslev P, McDonald IR, Iversen N, Henriksen K, Murrell JC (1999) Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. Appl Environ Microbiol 65:3312–3318

Horz HP, Yimba MT, Liesack W (2001) Detection of methanotroph diversity on roots of submerged rice plants by molecular retrieval of *pmoA, mmoX, mxaF*, and 16S rRNA and ribosomal DNA, including *pmoA*-based terminal restriction fragment length polymorphism profiling. Appl Environ Microbiol 67:4177–4185

Horz HP, Raghubanshi AS, Heye J, Kammann C, Conrad R, Dunfield PF (2002) Activity and community structure of methane-oxidising bacteria in a wet meadow soil. FEMS Microbiol Ecol 41:247–257

Hou S, Makarova KS, Saw JHW, Senin P, Ly BV, Zhou Z, Ren Y, Wang J, Galperin MY, Omelchenko MV, Wolf YI, Yutin N, Koonin EV, Stott MB, Mountain BW, Crowe MA, Smirnova AV, Dunfield PF, Feng L, Wang L, Alam M (2008) Complete genome sequence of the

extremely acidophilic methanotroph isolate V4, *Methylacidiphilum infernorum*, a representative of the bacterial phylum *Verrucomicrobia*. Biol Direct 3:26

Hou L-Y, Wang Z-P, Wang J-M, Wang B, Zhou S-B, Li L-H (2012) Growing season in situ uptake of atmospheric methane by desert soils in a semiarid region of northern China. Geoderma 189–190:415–422

Hutchens E, Radajewski S, Dumont MG, McDonald IR, Murrell JC (2004) Analysis of methanotrophic bacteria in Movile Cave by stable isotope probing. Environ Microbiol 6:111–120

Hütsch BW (1998) Methane oxidation in arable soil as inhibited by ammonium, nitrite, and organic manure with respect to soil pH. Biol Fertil Soils 28:27–35

Hyman MR, Wood PM (1983) Methane oxidation by *Nitrosomonas europaea*. Biochem J 212(1):31–37

Ishizuka S, Sakata T, Ishizuka K (2000) Methane oxidation in Japanese forest soils. Soil Biol Biochem 32:769–777

Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK (2008) Methane oxidation at 55 °C and pH 2 by a thermoacidophhhilic bacterium belonging to the *Verrucomicrobia* phylum. Proc Natl Acad Sci USA 105:300–304

Jacinthe PA, Lal K (2006) Spatial variability of soil properties and trace gas fluxes in reclaimed mine land of southeastern Ohio. Geoderma 136:598–608

Jehmlich N, Schmidt F, Taubert M, Seifert J, Bastida F, von Bergen M, Richnow HH, Vogt C (2010) Protein-based stable isotope probing. Nat Proto 5:1957–1966

Jensen S, Olsen RA (1998) Atmospheric methane consumption in adjacent arable and forest systems. Soil Biol Biochem 30:1187–1193

Jones RD, Morita RY (1983) Methane oxidation by *Nitrosococcus oceanus* and *Nitrosomonas europaea*. Appl Environ Microbiol 45:401–410

Khalil MI, Baggs EM (2005) CH_4 oxidation and N_2O emissions at varied soil water-filled pore spaces and headspace CH_4 concentrations. Soil Biol Biochem 35:1785–1794

Kip N, Dutilh BE, Pan Y, Bodrossy L, Neveling K, Kwint MP, Jetten MSM, Op den Camp HJM (2011) Ultra-deep pyrosequencing of *pmoA* amplicons confirms the prevalence of *Methylomonas* and *Methylocysti*s in Sphagnum mosses from a Dutch peat bog. Environ Microbiol Rep 3:667–673

Kirka JL, Beaudettea LA, Hartb M, Moutoglis P, Klironomos JN, Lee H, Trevors JT (2004) Methods of studying soil microbial diversity. J Microbiol Methods 58:169–188

Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, Bergamaschi P, Bergmann D, Blake DR, Bruhwiler L, Cameron-Smith P, Castaldi S, Chevallier F, Feng L, Fraser A, Heimann M, Hodson EL, Houweling S, Josse B, Fraser PJ, Krummel PB, Lamarque J-F, Langenfelds RL, Quéré CL, Naik V, O'Doherty S, Palmer PI, Pison I, Plummer D, Poulter B, Prinn RG, Rigby M, Ringeval B, Santini M, Schmidt M, Shindell DT, Simpson IJ, Spahni R, Steele LP, Strode SA, Sudo K, Szopa S, van der Werf GR, Voulgarakis A, van Weele M, Weiss RF, Williams JE, Zeng G (2013) Three decades of global methane sources and sinks. Nat Geosci 6:813–823

Kizilova A, Yurkov A, Kravchenko I (2013) Aerobic methanotrophs in natural and agricultural soils of European Russia. Diversity 5:541–556

Klemedtsson AK, Klemedtsson L (1997) Methane uptake in Swedish forest soil in relation to liming and extra N-deposition. Biol Fertil Soils 25:296–301

Knief C, Dunfield PF (2005) Response and adaptation of different methanotrophic bacteria to low methane mixing ratios. Environ Microbiol 7:1307–1317

Knief C, Lipski A, Dunfield PF (2003) Diversity and activity of methanotrophic bacteria in different upland soils. Appl Environ Microbiol 69:6703–6714

Kolb S, Knief C, Stubner S, Conrad R (2003) Quantitative detection of methanotrophs in soil by novel *pmoA*-targeted real-time PCR assay. Appl Environ Microb 69:2423–2429

Kolb S, Knief C, Dunfield PF, Conrad R (2005) Abundance and activity of uncultured methanotrophic bacteria involved in the consumption of atmospheric methane in two forest soils. Environ Microbiol 7:1150–1161

Kravchenko I, Boeckx P, Galchenko V, Van Cleemput O (2002) Short- and medium-term effects of NH4+ on CH4 and N2O fluxes in arable soils with a different texture. Soil Biol Biochem 34:669–678

Kravchenko IK, Semenov VM, Kuznetsova TV, Dulov LE, Semenova NA, Gal'chenko VF, Boeckx P, Van Cleemput O (2004) Methane oxidation and nitrogen transformations in gray forest soil. Eur Soil Sci 37:49–56

Kravchenko IK, Semenov VM, Kuznetsova TV, Bykova SA, Dulov LE, Pardini G, Gispert M, Boeckx P, Van Cleemput O, Gal'chenko VF (2005) Physicochemical and biological factors affecting atmospheric methane oxidation in gray forest soils. Microbiology 74:216–220

Kravchenko IK, Kizilova AK, Bykova SA, Men'ko EV, Gal'chenko VF (2010) Molecular analysis of high-affinity methane oxidizing enrichment cultures isolated from a forest biocenosis and agrocenoses. Microbiology 79:105–113

Kravchenko I, Sukhacheva M, Kizilova A (2014) Characterization of methanotrophic bacterial populations in natural and agricultural aerobic soils of the European Russia. Geophysical Res Abst 16:EGU2014–EG14349

Lau E, Ahmad A, Steudler PA, Cavanaugh CM (2007) Molecular characterization of methanotrophic communities in forest soils that consume atmospheric methane. FEMS Microbiol Ecol 60:490–500

Lau E, Nolan EJ IV, Dillard ZW, Dague RD, Semple AL, Wentzell WL (2015) High throughput sequencing to detect differences in methanotrophic *Methylococcaceae* and *Methylocystaceae* in surface peat, forest soil, and Sphagnum moss in Cranesville Swamp Preserve, West Virginia, USA. Microorganisms 3:113–136

Leckie SE (2005) Methods of microbial community profiling and their application to forest soils. Forest Ecol Manage 220:88–106

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. Eur J Soil Biol 37:25–50

Levine UY, Teal TK, Robertson GP, Schmidt TM (2011) Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane. ISME J 5:1683–1691

Lin J-L, Radajewski S, Eshinimaev BT, Trotsenko YA, McDonald IR, Murrell JC (2004) Molecular diversity of methanotrophs in Transbaikal soda lake sediments and identification of potentially active populations by stable isotope probing. Environ Microbiol 6:1049–1060

Lin JL, Joye SB, Scholten JCM, Schäfer H, McDonald IR, Murrell JC (2005) Analysis of methane monooxygenase genes in Mono Lake suggests that increased methane oxidation activity may correlate with a change in methanotroph community structure. Appl Environ Microbiol 71:6458–6462

Liu L, Greaver TL (2009) A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emission. Ecol Lett 12:1103–1117

Liu B, Morkved PT, Frostegård A, Bakken LR (2010) Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. FEMS Microbiol Ecol 72:407–417

Liu AC, Chou CY, Chen LL, Kuo CH (2015) Bacterial community dynamics in a swine wastewater anaerobic reactor revealed by 16S rDNA sequence analysis. J Biotechnol 194:124–131

Lüke C, Krause S, Cavigioli S, Greppi D, Lupotto E, Frenzel P (2010) Biogeography of wetland rice methanotrophs. Environ Microbiol 12:862–872

Lüke C, Frenzel P (2011) Potential of *pmoA* amplicon pyrosquencing for methanotroph diversity studies. Appl Environ Microbiol 77:6305–6309

Luo GJ, Kiese R, Wolf B, Butterbach-Bahl K (2013) Effects of soil temperature and moisture on methane uptake and nitrous oxide emissions across three different ecosystem types. Biogeosciences 10:3205–3219

Lynch MDJ, Neufeld JD (2015) Ecology and exploration of the rare biosphere. Nat Rev Microbiol 13:217–229

MacDonald JA, Skiba U, Sheppard LJ, Hargreaves KJ, Smith KA, Fowler D (1996) Soil environmental variables affecting the flux of methane from a range of forest, moorland and agricultural soils. Biogeochemistry 34:113–132

Malik S, Beer M, Megharaj M, Naidu R (2008) The use of molecular techniques to characterize the microbial communities in contaminated soil and water. Environ Int 34:265–276

Manefield M, Whiteley AS, Griffiths RI, Bailey M (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. Appl Environ Microbiol 68:5367–5373

Manucharova NA, Stepanov AL, Umarov MM (2001) Peculiarities of microbial transformation of nitrogen in water-stable aggregates of soils of different types. Eur Soil Sci 10:1261–1267

Martiny AC, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. ISME J 7:830–838

Maxfield PJ, Hornibrook ERC, Evershed RP (2008) Acute impact of agriculture on high-affinity methanotrophic bacterial populations. Environ Microbiol 10:1917–1924

McDonald IR, Bodrossy L, Chen Y, Murrell JC (2008) Molecular ecology techniques for the study of aerobic methanotrophs. Appl Environ Microbiol 74:1305–1315

Meixner FX, Yang WX (2006) Biogenic emissions of nitric oxide and nitrous oxide from arid and semi-arid land. In: D'Odorico P, Porporato A (eds) Dryland ecohydrology. Springer, Berlin, pp 233–255

Mohanty SR, Bodelier PLE, Floris V, Conrad R (2006) Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. Appl Environ Microbiol 72:21346–21354

Mosier A, Schimel D, Valentine D, Bronson K, Parton W (1991) Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature 350:330–332

Myrold DD, Zeglin LH, Jansson JK (2013) The potential of metagenomic approaches for understanding soil microbial processes. Soil Sci Soc Am J 78:3–10

Nazaries L, Tate KR, Ross JD, Singh J, Dando J, Saggar S, Baggs EM, Millard P, Murrell JC, Singh BK (2011) Response of methanotrophic communities to afforestation and reforestation in New Zealand. ISME J 5:1832–1836

Nicolaisen MH, Baelum J, Jacobsen CS, Sorensen J (2008) Transcription dynamics of the functional tfdA gene during MCPA herbicide degradation by *Cupriavidus necator* AEO106 (pRO101) in agricultural soil. Environ Microbiol 10:571–579

Park JR, Moon S, Ahn YM, Kim JY, Nam K (2005) Determination of environmental factors influencing methane oxidation in a sandy landfill cover soil. Environ Technol 26:93–102

Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MSM, Op den Camp HJM (2007) Methanotrophy below pH 1 by a new V*errucomicrobia* species. Nature 450:874–878

Priemé A, Christensen S (1997) Seasonal and spatial variation of methane oxidation in a Danish spruce forest. Soil Biol Biochem 29:1165–1172

Purkhold U, Pommerening-Röser A, Juretschko S, Schmid MC, Koops HP, Wagner M (2000) Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA s*equence analysis: implications for molecular diversity surveys. Appl Environ Microbiol 66:5368–5382

Radajewski S, Ineson P, Parekh NR, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403:646–649

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WI, Schouten S, Damsté JSS, Op den Camp HJM, Jetten MSM, Strous M (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440:918–992

Reaya DS, Nedwella DB, McNamara N, Ineson P (2005) Effect of tree species on methane and ammonium oxidation capacity in forest soils. Soil Biol Biochem 37:719–730

Roalkvam I, Jorgensen SL, Chen Y, Stokke R, Dahle H, Hocking WP, Lanzén A, Haflidason H, Steen IH (2011) New insight into stratification of anaerobic methanotrophs in cold seep sediments. FEMS Microbiol Ecol 78:233–243

Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, Daroub SH, Camargo FA, Farmerie WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 4:283–290

Roslev P, Iversen N, Henriksen K (1997) Oxidation and assimilation of atmospheric methane by soil methane oxidizers. Appl Environ Microbiol 63:874–880

Roslev P, Iversen N (1999) Radioactive fingerprinting of microorganisms that oxidize atmospheric methane in different soils. Appl Environ Microbiol 65:4064–4070

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. Global Change Biol 1:77–91
Schindlbacher A, Zechmeister-Boltenstern S, Butterbach-Bahl K (2004) Effects of soil moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils. J Geophys Res 109:D17
Schnell S, King GM (1996) Responses of methanotrophic activity in soils and cultures to water stress. Appl Environ Microbiol 62:3203–3209
Seghers D, Top EM, Reheul D, Bulcke R, Boeckx P, Verstraete W, Sicilliano SD (2003) Long-term effects of mineral versus organic fertilizers on activity and structure of the methanotrophic community in agricultural soils. Environ Microbiol 5:867–877
Seghers D, Verthe K, Reheul D, Bulcke R, Siciliano SD, Verstraete W, Top EM (2006) Effect of longerm herbicide applications on the bacterial community structure and function in an agricultural soil. FEMS Microb Ecol 46:139–146
Semenov VM, Kravchenko IK, Kuznetsova TV, Semenova NA, Bykova SA, Dulov LE, Gal'chenko VF, Pardini G, Gispert M, Boeckx P, Van Cleemput O (2004) Seasonal dynamics of atmospheric methane oxidation in gray forest soils. Microbiology 73:356–362
Semenov V, Kuznetsova T, Semenov A, Kravchenko I (2013) Methane emission and consumption in Russian gray forest agricultural soil: monthly, daily and diurnal variations and effect of mineral and organic fertilization. World Appl Sci J 24:528–533
Serrano-Silva N, Sarria-Guzman Y, Dendooven L, Luna-Guido M (2014) Methanogenesis and methanotrophy in soil: a review. Pedosphere 24:291–307
Sharma S, Radl V, Hai B, Kloos K, Mrkonjic M, Engel FM, Schauss K, Schloter M (2007) Quantification of functional genes from prokaryotes in soil by PCR. J Microbiol Methods 68:445–452
Shrestha M, Abraham WR, Shrestha PM, Noll M, Conrad R (2008) Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of *pmoA* gene and stable isotope probing of phospholipid fatty acids. Environ Microbiol 10:400–412
Shvaleva A, Siljanen HM, Correia A, Costa e Silva F, Lamprecht RE, Lobo-do-Vale R, Bicho C, Fangueiro D, Anderson M, Pereira JS, Chaves MM, Cruz C, Martikainen PJ (2015) Environmental and microbial factors influencing methane and nitrous oxide fluxes in Mediterranean cork oak woodlands: trees make a difference. Front Microbiol 6:1104
Singh BK, Tate KR, Kolipaka G, Hedley CB, Macdonald CA, Millard P, Murrel JC (2007) Effect of afforestation and reforestation of pastures on the activity and population dynamics of methanotrophic bacteria. Appl Environ Microbiol 73:5153–5161
Slobodova NV, Kolganova NV, Boulygina ES, Kuznetsov BB, Tourova TP, Kravchenko IK (2006) Comparative characterization of methanotrophic enrichments by serological and molecular methods. Microbiology 75:336–342
Smith CJ, Osborn AM (2009) Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology FEMS Microb Ecol 67:6–20
Smith KA, Dobbie KE, Ball BC, Bakken R, Sitaula BK, Hansen S, Brumme R, Borken W, Christensen S, Prieme A, Fowler D, MacDonald A, Skiba U, Klemedtsson I, Kasimir-Klemedtsson A, Degorska A, Orlanski P (2000) Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. Global Change Biol 6:791–803
Smith KA, Ball T, Conen F, Dobbie KE, Massheder J, Re AY (2003) Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eur J Soil Sci 54:779–791
Steinkamp R, Butterbach-Bahl K, Papen H (2001) Methane oxidation by soils of an N limited and N fertilized spruce forest in the Black Forest, Germany. Soil Biol Biochem 33:145–153
Steudler PA, Bowden RD, Melillo JM, Aber JD (1989) Influence of nitrogen fertilization on methane uptake in temperate forest soils. Nature 341:314–316
Stevenson D, Dentener F, Schultz M, Ellingsen K, van Noije T, Wild O, Zeng G, Amann M, Atherton C, Bell N, Bergmann D, Bey I, Butler T, Cofala J, Collins W, Derwent R, Doherty R, Drevet J, Eskes H, Fiore A, Gauss M, Hauglustaine D, Horowitz L, Isaksen I, Krol M, Lamarque

JF, Lawrence M, Montanaro V, Muller J-F, Pitari G, Prather M, Pyle J, Rast S, Rodriguez J, Sanderson M, Savage N, Shindell D, Strahan S, Sudo K, Szopa S (2006) Multimodel ensemble simulations of present-day and near-future tropospheric ozone. J Geophys Res 111:1–23

Stoecker K, Bendinger B, Schoning B, Nielsen PH, Nielsen JL, Baranyi C, Toenshoff ER, Daims H, Wagner M (2006) Cohn's *Crenothrix* is a filamentous methane oxidizer with an unusual methane monooxygenase. Proc Natl Acad Sci 103:2363–2367

Striegl RG (1993) Diffusional limits to the consumption of atmospheric methane by soils. Chemosphere 26:715–720

Strieg RG, McConnaughey TA, Thorstenson DC, Weeks EP, Woodward JC (1992) Consumption of atmospheric methane by desert soils. Nature 357:145–147

Sundh I, Borga P, Nilsson M, Svensson BH (1995a) Estimation of cell numbers of methanotrophic bacteria in boreal peatlands based on analysis of specific phospholipid fatty acids. FEMS Microbiol Ecol 8:103–112

Sundh I, Mikkela C, Nilsson M, Svensson BH (1995b) Potential aerobic methane oxidation in a Sphagnum-dominated peatland-controlling factors and relation to methane emission. Soil Biol Biochem 27:829–837

Suwanwaree P, Robertson GP (2005) Methane oxidation in forest, successional, and no-till agricultural ecosystems. Soil Sci Soc Am J 69:1722–1729

Tamai N, Takenaka C, Ishizuka S, Tezuka T (2003) Methane flux and regulatory variables in soils of three equal-aged Japanese cypress (*Chamaecyparis obtusa*) forests in central Japan. Soil Biol Biochem 35:633–641

Tate KR, Ross DJ, Scott NA, Rodda NJ, Townsend JA, Arnold GC (2006) Post-harvest patterns of carbon dioxide production, methane uptake and nitrous oxide production in a *Pinus radiata* D. Don plantation. Forest Ecol Manage 228:40–50

Tlustos P, Willison TW, Baker JC, Murphy DV, Pavlikova D, Goulding KWT, Powlson DS (1998) Short-term effects of nitrogen on methane oxidation in soils. Biol Fertil Soils 28:64–70

Trotsenko YA, Murrell JC (2008) Metabolic aspects of aerobic obligate methanotrophy. Adv Appl Microbiol 63:183–229

Vecherskaya MS, Galchenko VF, Sokolova EN, Samarkin VA (1993) Activity and species composition of aerobic methanotrophic communities in tundra soils. Curr Microbiol 27:181–184

Von Fischer JC, Hedin LO (2007) Controls on soil methane fluxes: tests of biophysical mechanisms using stable isotope tracers. Global Biogeochem Cycles 21:1–9

Vorobev AV, Baani M, Doronina NV, Brady AL, Liesack W, Dunfield PF, Dedysh SN (2011) *Methyloferula stellata* gen. nov., sp. nov., an acidophilic, obligately methanotrophic bacterium that possesses only a soluble methane monooxygenase. Int J Syst Evol Microbiol 10:2456–2463

Wahlen M (1993) The global methane cycle. Ann Rev Earth Planet Sci 21:407–426

Wang Z-P, Ineson P (2003) Methane oxidation in a temperate coniferous forest soil: effects of inorganic N. Soil Biol Biochem 35:427–433

Zhang W, Mo J, Zhou G, Gundersen P, Fang Y, Lu X, Zhang T, Dong S (2008) Methane uptake responses to nitrogen deposition in three tropical forests in southern China. J Geophys Res 113:1–10

Zhou XQ, Wang YF, Huang XZ, Hao YB, Tian JQ, Wang JZ (2008) Effects of grazing by sheep on the structure of methane-oxidizing bacterial community of steppe soil. Soil Biol. Biochem 40:258–261

Chapter 11
Microbial-Mediated Lindane Bioremediation

Siddharth Boudh, Shashank Tiwari, and Jay Shankar Singh

Abstract Hexachlorocyclohexane (HCH), commonly known as lindane, is the term which collectively identifies the eight isomers of the HCH. The lindane has only insecticidal properties and also considered as most toxic isomer of the HCH. The lindane has been listed as a persistent organic pollutant (POPs) under Stockholm Convention on June 29, 2005. More than 52 countries not only globally banned for its formulation but also use of lindane in any form. After knowing facts regarding its toxicity, persistence nature, and bioaccumulation, some countries are still producing and exporting the lindane on large scale. The countries involved in lindane formulation are creating dumping sites which are the major source of lindane contamination to the adjoining area. The lindane deposited in the cultivated soils is also affecting to the non-target organisms. Apart from this, scientists start working on its degradation and find out that bioremediation is the easiest, cheapest, and safest way to remove the lindane from contaminated sites. Bioremediation by the microalgae could help in decontaminating polluted aquatic ecosystems and in cleaning the effluents before they are discharged into aquatic systems. Many microorganisms show tremendous potential in lindane degradation. The present review article describes about all known possible lindane-degrading microorganisms used for its bioremediation and also concise advanced techniques used for this purpose.

Keywords Bioremediation • Lindane • Microbes • Nanoparticles • Persistent organic pollutant

S. Boudh • S. Tiwari
Department of Environmental Microbiology, School for Environmental Sciences,
B.B. Ambedkar (Central) University, Raibarelly Road, Lucknow, Uttar Pradesh 226025, India

J.S. Singh (✉)
Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@bbau.ac.in

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_11

11.1 Introduction

Pesticides are major agro-input in our modern agriculture and are used to reduce the pest attack and increase crop yield with uncontrolled measures because of illiteracy, cheap cost, and not but the least for producing high yield of crops at any cost. The most prominent pesticides in use are organochlorines (DDT, Lindane, Aldrin, Dieldrin, etc.). Residues of organochlorine pesticides are integral part of our environment. Because of their strong lipophilic and persistent nature, they tend to accumulate in different trophic level in various natural and man-made ecosystems and causing adverse/lethal affects on livings.

The remediation of contaminated sites is carried out by conventional methods such as excavation, landfills, incineration, stabilization, and vitrification (Fuentes et al. 2010a, b). However, microbial diversity may offers an eco-friendly option for the mineralization of pesticides into less toxic metabolites (Singh et al. 2011a, b, c; Pandey et al. 2014; Singh et al. 2016). Bioremediation, which includes the gainful utilization of microorganisms for the biodegradation of target pollutants, is a potential technique for the biological treatment of industrial wastes and contaminated soils (Crawford and Crawford 1996; Alexander 1999). Bio-augmentation is the intensification of pollutant dissipation by the addition of appropriate microbes by inoculation and is widely recognized as a promising technique for the enhanced bioremediation of persistent chemicals and pesticides. In this review, an effort is made to summarize the current status of microbial-mediated lindane bioremediation.

11.2 Lindane

Lindane or Hexachlorocyclohexane (HCH) is a cyclic, saturated hydrocarbon that exists primarily in four isomeric forms: α-, β-, γ-, and δ-HCH. The γ-isomer of HCH (commonly known as lindane) and technical HCH (which includes α-, β-, γ-, and δ-isomers) have been used extensively against agricultural pests and in malaria health programs, worldwide. Initially, lindane was registered for uses in the different sector as preplant treatment of seeds for certain grain and vegetable crops, protection against insect pests, veterinary sector to treat livestock and their bedding, public health sector as a treatment for external parasites such as head lice and scabies, forestry sector to protect trees and seedlings from various insect pests, and garden use for the treatment of pets.

For production of each tone of lindane, about 8–12 tonnes of wastes are generated (generally enriched in the α- and β-isomers). Thus these production residues were deposited in an uncontrolled manner around the various production sites (Vijgen et al. 2006; Acat and Panna 2008). Because of their toxicity, lipophilic nature, long-range transport capability through wind, persistence behavior and easily bio-accumulative in food chain, α-, β- and γ- isomers are considered as most hazardous organic chemicals. Table 11.1 shows preliminary estimated data about quantities of stored/deposited HCH waste isomers gathering information from HCH

Table 11.1 HCH isomers present in contaminated soils of different countries

S.no.	Country	HCH (tonnes)
1.	Turkey	23,500
2.	Macedonia	35,500
3.	Romania	310,000
5.	Slovakia	26,000
6.	Poland	35,000
7.	Germany	373,000
9.	France	330,000
10.	Spain	200,000
15.	USA	65,000
16.	Brazil	50,000
17.	South Africa	70,000
18.	India	56,000
19.	Soviet Union	250,000
20.	China	91,200
22.	Japan	76,000

The data has been modified from Vijgen et al. (2011)

Table 11.2 Health problems due to HCH contaminations

HCH isomers	Health effects
A	Hepatic nodules and hepatocellular carcinomas
B	Environmental estrogen
γ	Stimulates and damages central nervous system at high doses
Δ	Inadequate data from animal assay
Technical grade	Carcinogenicity observed in mice

The data has been modified from Willett et al. (1998)

& pesticide forums and from literature survey (Vijgen et al. 2011). Health problems related to HCH isomers are summarized in Table 11.2.

In October 2008 during Stockholm Convention (Annex-A) the lindane has been listed as persistent organic pollutants (Vijgen et al. 2011). Now lindane can only be used in human health pharmaceutical for the control of head lice and scabies as a second-line treatment (UNEP 2009). India has a total installed capacity of lindane (technical) production of 1300 tonnes per annum, with two companies producing: Kanoria Chemicals and Industries Limited (Sonebhadra district) with a capacity of 300 tonnes per annum and India Pesticides Limited (Lucknow) with a 300 tonnes per annum (Abhilash and Singh 2009a, b). Now India Pesticides Limited, Lucknow is the only operating plant worldwide. The recent lindane production technology with processing the lindane isomers into TCB and HCl, which less toxic in nature (Crop Life 2006). India Pesticides Limited also offers products from the recycling of HCH-commercial grade trichlorobenzene,1,2,4-TriCBz and onward reaction products like 2,4,5-trichloroaniline,1,2,4-trichloro-5-nitrobenzene, and even hexachlorobenzene.

There is an urgent need for the on-site remediation of contaminated soil or stock-piles of lindane in order to prevent the migration of these HCH isomers to other

environmental compartment and food chain. It is now well accepted that microbes have enormous catabolic potential and wide range of survival in harsh environment. Therefore, microbes can be exploited as efficient tool for removal of persistent pollutants like lindane from the contaminated sites (Singh et al. 2011a).

11.3 Microorganisms Involved in Bioremediation of Lindane

Lindane residues from "muck" due to rain water, wind and because of illegal discharge in water bodies can enter the environment and cause toxicity (Willett et al. 1998; ATSDR 1999). Lindane residues have been reported to found in bovine milk (Nag and Raikwar 2008), milk products (Pandit and Sahu 2002), meat (Aulakh et al. 2006), river water (Kaushik et al. 2007), groundwater (Shukla et al. 2006), soil (Abhilash et al. 2008), packed water bottles (CSE Report 2003), fish (Amaraneni and Pillala 2001), human blood (Dhananjayan et al. 2012), butter and ghee (Battu et al. 2004), honey sample (Choudhary and Sharma 2008), vegetables (Bhanti and Taneja 2004), breast milk (Kalra et al. 2003), and maternal and cord blood (Pathak et al. 2008).

For this reason, scientists have isolated and characterized the microorganisms having capability for the degradation of lindane and other HCH isomers under anaerobic (MacRae et al. 1969; Jagnow et al. 1977; Van Eekert et al. 1998; Van Doesburg et al. 2005) and aerobic conditions (Senoo and Wada 1989; Sahu et al. 1990; Thomas et al. 1996; Gupta et al. 2000; Manonmani et al. 2000; Okeke et al. 2002; Boltner et al. 2005; Kumar et al. 2005). A brief description of microorganism that degrades lindane has been listed in Tables 11.3.

11.3.1 Actinomycetes

Actinomycetes are very successful in lindane bioremediation as these microorganisms are well adapted to the various lindane-contaminated sites (Shelton et al. 1996; Ravel et al. 1998). Pesticide-degrading actinomycetes genera are *Arthrobacter, Brevibacterium, Clavibacter, Corynebacterium, Micromonospora, Mycobacterium, Nocardia, Nocardioides, Rhodococcus,* and *Streptomyces* (De Schrijver and De Mot 1999). Benimeli et al. (2003, 2006, 2007) and Benimeli (2004) isolated wild-type *Streptomyces* strains able to tolerate and remove lindane from river sediments and other local contaminated sites from Tucuman, Argentina. *Streptomyces* sp. M7 was found able to grow in sterile soil with different initial pesticide concentrations (100, 150, 200, and 300 μg/kg) (Benimeli et al. 2008). Cuozzo et al. (2009) detected dechlorinase activity and lindane catabolism by *Streptomyces* sp. M7. He also demonstrated that synthesis of dechlorinase in *Streptomyces* sp. M7 was induced when the microorganism was grown in the presence of lindane as only carbon source. He also reported the release of c-2,3,4,5,6-pentachlorocyclohexene and 1,3,4,6-tetrachloro-1,4-cyclohexadiene, the first and second products in lindane degradation by

Table 11.3 An overview of lindane-degrading microorganism

Microbe	Degradation nature	Initial HCH concentration	Matrix used	Intermediate metabolites	Removal rate (%)	References
Clostridium sp.	Anaerobic	3.7 mg/L	Liquid	Chloride ion	99 % in 27 h	MacRae et al. (1969)
Clostridium rectum	Anaerobic	0.017 mg/L	Liquid	Monochlorobenzene, γ-3,4,5,6-TCCH	100 % in 3 h	Ohisa et al. (1980)
Pseudomonas aeruginosa ITRC-5	Aerobic	2000 mg/kg	Soil slurry	γ-PCCH, 1,2,4-TCB, CHQ	98 % in 15 days	Manickam et al. (2008)
Pseudomonas putida	Aerobic		Liquid	γ-PCCH, γ-TCCH		Matsumura et al. (1976)
Sphingomonas paucimobilis UT26	Aerobic	4 mg/L	Liquid	γ-PCCH		Imai et al. (1989)
Microbacterium sp. ITCR 1	Aerobic	200 mg/kg	Soil slurry	2,5-DCP	96 % in 28 days	Manickam et al. (2006a)
Arthrobacter citreus BI-100	Aerobic	100 mg/L	Liquid	γ-PCCH, TCCH, TCCD, 2-chlorophenol, phenol, catechol	100 % in 8 h	Datta et al. (2000)
Desulfovibrio gigas, *Desulfococcus multivorans*	Anaerobic	7 mg/L	Liquid	Benzene, chlorobenzene	100 % in 19 days	Badea et al. (2009)

Notes: *γ-HCH* hexachlorocyclohexane, *γ-PCCH* γ-pentachlorocyclohexene, *1,2,4-TCB* 1,2,4-trichlorobenzene, *1,4-DCB* 1,4-dichlorobenzene, *2,5-DCBQ* 2,5-dichlorobenzoquinone, *2,5-DCP* dichlorophenol, *CHQ* chlorohydroquinone, *TCCD* trichlorocyclohexadiene, *TCCH* tetrachlorocyclohexene, *TCCOL* tetrachlorocyclohexenol

Streptomyces sp. M7 according to the catabolic pathway proposed by Nagata et al. (2007). Fuentes et al. (2010a, b, 2011) isolated actinomycetes, belonging to the *Streptomyces* and *Micromonospora* genera from sites contaminated with organochlorine pesticides. These strains release chloride ions as a result of lindane degradation. These results favor application of actinomycetes as potential agents for bioremediation of polluted environments with different organochlorine pesticides.

De Paolis et al. (2013) isolated *Arthrobacter fluorescens* and *A. giacomelloi* having ability to grow in a mineral salt medium containing α-, β-, or γ-HCH (100 mg/L) as sole source of C. Although both bacteria were able to metabolize the HCHs, but *A. giacomelloi* was found most effective when incubated with pentachlorocyclohexenes and tetrachlorocyclohexenes for 72 hrs.

11.3.2 Algae

Microalgae-mediated bioremediation could help in decontaminating polluted lakes and contaminated effluents before they are discharged into aquatic systems and has been considered as a low cost and naturally renewable technology. Kobayashi and Rittmann (1982) observed the capacity of some algae (*Chlorella vulgaris* and *Chlamydomonas reinhardtii*) not only to bioaccumulate but also to transform lindane to PCCH under aerobic conditions. *Anabaena* sp. and *Nostoc ellipsosporum* transformed lindane first to γ-pentachlorocyclohexene (γ-PCCH) and then to a mixture of chlorobenzenes. This process was co-metabolic and depended on the presence of nitrate (Kuritz and Wolk 1995). Kuritz et al. (1997) screened 15 strains of wild-type cyanobacteria (*Anabaena* sp. PCC7120, *Anabaena* sp. P30, *Calothrix* sp. ATCC29112, *Fischerella* sp. CALU926, *F. musciola* UT1829, *Nostoc ellipsosporum*, *N. muscorum* UT387, *N. parmeioides* UT162, *Nostoc* sp. GSV39, *Nostoc* sp. GSV40, *Nostoc* sp. GSV236, *Phormidium uncinatum*, *Plectonema* sp., *P. boryanum*, *Synechococcus* PCC7942) able to degrade lindane with different efficiencies. The cyanobacterium *Anabaena* PCC7119 can tolerate lindane conc. up to 5 ppm, without significant changes in the photosynthetic vitality index of the cells (Bueno et al. 2004).

El-Bestawy et al. (2007) isolated lindane-degrading cyanobacterial species from the two Egyptian Lakes (Qaroun and Mariut). Growth inhibition or stimulation percentage of lindane removal efficiency (RE) was also calculated. The order of lindane tolerence among Qaroun lake members was *Oscillatoria* sp. > *Synechococcus* sp. > *Nodularia* sp. > *Nostoc* sp. > *Cyanothece* sp. > *Synechococcus* sp, while among Mariut lake members the order of lindane tolerance was *Microcystis aeruginosa* MA1 > *Anabaena cylindrica* > *M. aeruginosa* MA15 > *A. spiroides* > *A. flosaquae*. Evidences suggest that mutation at one locus in the chromosome of bacteria can change and make adaptation to the diverse hostile contaminated sites (Flores-Moya et al. 2005; Costas et al. 2007, 2008; Lopez-Rodas et al. 2008a) as well pathogenic potentials (Costas et al. 2001; Lopez-Rodas et al. 2001, 2007; Garcia-Villada et al. 2004). Lindane-resistant cells of microalgae *Scenedesmus intermedius* (Clorophyta) were able to eliminate lindane (5–40 mg/L) efficiently (González et al. 2012).

11.3.3 Bacteria

It was initially believed that lindane biodegradation is largely an anaerobic process, and variable levels of anaerobic degradation of α-, β-, γ-, and δ-HCH. During early stage of lindane degrading investigations it was assumed that anaerobic microorganisms use lindane as a sole carbon and energy source. But later studies demonstrated that lindane is used as an electron acceptor under anaerobic conditions rather than as a carbon source. MacRae et al. (1969) reported degradation of lindane by anaerobic *Clostridium* sp. (Ohisa and Yamaguchi 1978). Other isolates capable of degrading HCH isomers under anaerobic conditions are *C. sphenoides* (Heritage and MacRae 1977), *C. butyricum*, and *C. pasteurianum* (Jagnow et al. 1977).

Francis et al. (1975) reported lindane degradation by *Escherichia coli* isolated from rat feces. About 10 % of the added lindane was metabolized by the bacterium in trypticase soy broth where the lindane was used as pesticide. Members of the family Sphingomonadaceae appear to have an important role in aerobic lindane degradation. The lindane-degrading species of *Sphingobium japonicum* UT26 (Imai et al. 1989), *S. francense* (Dogra et al. 2004), and *S. indicum* B90A (Sahu et al. 1990) were isolated respectively from Japan, France, and Indian soils. The pathway for the degradation of lindane has been comprehensively worked out in the bacterium *S. paucimobilis* UT26 and the genes for its different enzymes have been characterized (Nagata et al. 1999).

The catabolic *lin* genes associated with the degradation of lindane were initially discovered in *S. japonicum* UT26 (Nagata et al. 1999). Six structural *lin* genes (*linA*–*linF*) (Imai et al. 1991; Nagata et al. 1993, 1994; Miyauchi et al. 1998, 1999; Endo et al. 2005) and one regulatory gene (*linR*) (Miyauchi et al. 2002) of *S. japonicum* UT26 are reported in complete mineralization of lindane. In addition, a *linX* gene, encoding a protein that has activity similar to that of *linC*, is also characterized (Nagata et al. 1994). The *linA*-encoded HCH dehydrochlorinase mediates the first two steps of dehydrochlorination of lindane. In addition to mediating the second step in the degradation of lindane in *S. japonicum* UT26, *linB* transforms β-HCH to 2,3,4,5,6-pentachlorocyclohexanol (PCHL) (Nagata et al. 2005). PCHL has lower hydrophobicity and lower chemical stability than β-HCH and the bacteria that degrade β-HCH completely by a combination of biological pathways (Ceremonie et al. 2006).

Flavobacterium sp., *Pseudomonas* sp., and *Acromobacter* sp. isolated from the gut of earthworms treated with lindane were capable of degrading α-, β-, and γ-isomers of HCH (Ramteke and Hans 1992). Other lindane-degrading bacteria such as *Citrobacter freundii* (Jagnow et al. 1977), *Desulfovibrio gigas*, *D. africanus*, *Desulfococcus multivorans* (Boyle et al. 1999), and a *Dehalobacter* sp. (van Doesburg et al. 2005) have been also isolated. Nalin et al. (1999) isolated a new strain of *Rhodanobacter lindaniclasticus* which degraded technical grade HCH under aerobic condition. Datta et al. (2000) reported the growth characteristics and degradation of the aerobic bacterial strain *A. citreus* BI-100 in mineral salts medium with lindane (100 mg/L) as the sole source of carbon. Gupta et al. (2001) reported the degradation of lindane by *Alcaligenes faecalis* isolated from agricultural fields.

Bacillus circulans and *B. brevis*, isolated from soil contaminated with lindane and acclimatized to different concentrations of lindane, degraded 80 % of γ-HCH (5 μg/mL) within 8 days (Gupta et al. 2000). The reductions of lindane by *Lactobacillus plantarum* were 27.9 and 40.0 %, respectively, in TSB and MSM without nitrite addition, or 38.4 and 48.4 % in the same media with nitrite addition (Abou-Arab 2002). Gram-negative *Pandoraea* sp. substantially degraded lindane under aerobic conditions at concentrations of 10–200 mg/L lindane in liquid cultures. After 8 weeks of incubation at pH 9.0 in liquid culture, 89.9 % of the lindane declined at an initial concentration of 150 mg/L (Okeke et al. 2002). The aerobic biodegradation of lindane by *B. thiooxidans* bacteria has been reported from sediment at a polluted site on the Suquia River, Cordoba, Argentina (Pesce and Wunderlin 2004). *B. thiooxidans* were able to degrade lindane after 3 days of growth. About 12 novel lindane-degrading bacterial strains have been isolated from lindane-contaminated sites at Chemnitz in Germany (Boltner et al. 2005) and Bilbao in northern Spain (Mohn et al. 2005). *P. paucimobilis* isolated from paddy field rhizosphere soil had ability to degrade lindane (Sahu et al. 1990). About 98 % of lindane was aerobically degraded by *S. paucimobilis* after 12 days of incubation (Johri et al. 1998). *S. ummariense* sp. nov. was isolated from an lindane dump site located in the northern part of India (Singh and Lal 2009). *P. aeruginosa* ITRC-5 can degrade α-, β-, γ-, and δ-HCH, in both liquid culture and contaminated soils (Kumar et al. 2005). Incubation of "muck" with *P. aeruginosa* ITRC-5 under optimized conditions favors the substantial degradation of HCH isomers (Chaudhary et al. 2006). Bioconversion and biological growth kinetics of *P. aeruginosa* degrading technical HCH in batch process has been seen under aerobic condition by Lodha et al. (2007). *Microbacterium* sp. ITRC1 has the capacity to degrade all four major isomers of HCH present in both liquid cultures and aged contaminated soil (Manickam et al. 2006). For the first time a *Xanthomonas* sp. was isolated from a contaminated soil, utilized lindane as sole carbon and energy source by successive dechlorination (Manickam et al. 2007).

A yellow-pigmented, lindane-degrading bacterium *S. quisquiliarum* P25 (T) was isolated from a lindane dumping site located in the northern part of India (Bala et al. 2009). Dadwal et al. (2009) reported lindane degradation capability of *S. chinhatense* IP26, *S.* UM2*, S.* HDU05 and *S*. UM1, *S.* F2*, S.* HDIP04. Maximum degradation of lindane by *Azotobacter chroococcum* JL 102 was recorded at 10 ppm lindane concentration in Jensen's medium. A pot culture experiment showed that the lindane degradation potential of *Azotobacter* sp. significantly increased when incubated for 8 weeks (Anupama and Paul 2010). During experiments from marine sediments, Boyle et al. (1999) demonstrated lindane biodegradation under sulfate reducing conditions with short chain fatty acids serving as the carbon and energy source with the release of chlorobenzene as by-products. Pure cultures of *Desulfovibrio gigas*, *D. africanus*, and *D. multivorans* were also able to dehalogenate lindane to benzene and chlorobenzene (Boyle et al. 2010). Zheng et al. (2011) reported degradation of lindane by *Sphingobium* strains (*S. indicum* B90A, *S. japonicum* UT26, *S. francense Sp*+) at low temperature (4 °C). Further, *S. lactosutens* sp. (Kumari et al. 2009), *Novosphingobium lindaniclasticum* sp. (Saxena

et al. 2012) and *S. baderi* LL03(T) sp. isolated from India, also showed lindane degradation potential (Kaur et al. 2012). While *S. czechense* sp. nov. and *N. barchaimii* sp. nov. were isolated from lindane-contaminated soil at Spolana Neratovice, Czech Republic (Niharika et al. 2012a, b). Also *Pseudoxanthomonas indica* sp. nov. (Kumari et al. 2010), *Flavobacterium ummariense* sp. nov. (Lata et al. 2011), *Pontibacter lucknowensis* sp. nov. (Dwivedi et al. 2012), *Pontibacter ramchanderi*, and *P. indicus* sp. nov. (Singh et al. 2013a, b) were isolated from a lindane dump site in Lucknow, India. After the above discussion it may be deduced that bacteria are one of the potential microbial agents that may be exploited for removal and decontamination of lindane-contaminated sites. Though a number of experiments have been conducted on white rot fungi mediated lindane remediation. However, there is need to isolate and identify the more efficient indigenous fungal strains having the potential capability to degrade lindane at lindane dumping sites.

11.3.4 Fungi

Most of the lindane-degrading fungi (used lindane as source of carbon and energy) known to date are the members of the family of white rot fungi but few nonwhite rot fungi has also been reported to degrade the lindane. The lindane biodegradation is accomplished with the action of extracellular oxidative enzymes such as laccase, manganese peroxidase, and lignin peroxidase produced by the fungus to decompose woody substrates (Rigas et al. 2005; McErlean et al. 2006). Bumpus et al. (1985) published the first report on the biodegradation of lindane by white rot fungi *Phanerochaete chrysosporium*. Mougin et al. (1997) reported the enhanced mineralization in soils supplemented with lindane by *Phanerochaete* sp. and the fungus seemed to modify lindane degradation pathway by increasing the conversion of volatile intermediates to CO_2. Biodegradation of lindane up to 85–95 % by white rot fungi such as *Pleurotus ostreatus, P. sajor-caju*, and *Trametes hirsutus* has been reported (Arisoy and Kolankaya 1997; Singh and Kuhad 1999; Papadopoulou et al. 2006). Singh and Kuhad (1999) investigated that lindane degradation ability of the white rot fungus *Trametes hirsutus* in liquid culture was less than *T. hirsutus*. Further, the lindane degradation capability of white rot fungi, *Cyathus bulleri* and *P. sordid* was found more than *P. sordida* (Singh and Kuhad 2000).

About 82 % degradation of lindane has been reported in batch cultures and about 81 % degradation was noted in packed bed reactor (Tekere et al. 2001). Bioremediation process was evaluated in polypore fungus, *Ganoderma australe*, in mixtures of a sandy soil and wheat straw doped with lindane (Rigas et al. 2007). Maximal lindane degradations of 94.5 % were attained after 30 days for lindane by the white rot fungus *Bjerkandera adusta* in a slurry batch bioreactor (Quintero et al. 2007). The lindane was degraded between 15.1 and 70.8 % by six of the nine fungal species, *B. adusta, P. ciliatus, L. tigrinus, S. hirsutum, P. eryngii,* and *I. lacteus* (Quintero et al. 2008). Biodegradation of lindane by *Phycomyceteous* and *Conidiobolus*, a nonwhite rot fungus, was reported by Nagpal et al. (2008). The fungus completely degraded lindane on the fifth day in the culture medium. The

bracket-like polypore fungus, *Ganoderma australe*, was selected for its potential to degrade lindane in liquid-agitated sterile cultures. The maximum lindane biodegradation (3.11 mg/g biomass) was obtained with addition of nitrogen supplements during 5 days of cultivation time (Dritsa et al. 2009). Two *Fusarium* species (*F. poae* and *F. solani*) isolated from the pesticide-contaminated soil showed better degradability of lindane (Sagar and Singh 2011). The fungal strain *F. verticillioides* AT-100 isolated from *Agave tequilana* leaves can degrade lindane (50 mg/L) after 7 days of incubation and utilize lindane as sole source of carbon and energy (Guillen-Jimeneza et al. 2012).

Salam and Das (2013) isolated four yeast strains on the basis of their lindane degradation ability. Among them, *Candida* sp. VITJzN04 showed the maximum potential for lindane degradation in solid as well as liquid media followed by *Rhodotorula* sp. VITJzN03, *Pseudozyma* sp. VITJzN01, and *Cintractia sorghi* VITJzN02. Degradation of lindane when grown at higher concentration (600 mg/L) by yeasts *Candida* VITJzN04 has been found within 6 days. Chemo-stimulation due to H_2O_2 addition in the mineral medium showed 32 % enhancement of lindane degradation within 3 days. In addition, involvement of the enzymes viz. dechlorinase, dehalogenase, dichlorohydroquinone reductive dechlorinase, lignin peroxidase, and manganese peroxidase was noted during lindane degradation (Salam and Das 2013).

Bio-augmentation (addition of some other microbes in the lindane-contaminated sites) may be a better option to enhance the in situ degradation of lindane by the naturally growing indigenous microflora (bacteria, fungi, etc.) of the soils. Mertens et al. (2006) encapsulated single HCH-degrading isolate, *Sphingomonas* sp. γ1–7, into open-ended silicone tubes and around half of the γ-HCH degradation was achieved in the experiment. Raina et al. (2008) conducted a bio-augmentation experiment by immobilizing *Sphingobium indicum* B90A on corncob powder and inoculating it to remove mixture of HCH isomers from pits of transplanted contaminated soil and agricultural site and after incubation about 80 % of the α- and γ-HCH were removed. Though a number of experiments have been conducted on white rot fungi mediated lindane remediation. However, there is need to isolate and identify the more efficient indigenous fungal strains having the potential capability to degrade lindane at lindane dumping sites.

11.4 Plant-Microbe Association in Lindane Remediation

Rhizoremediation (degradation of toxicants by microorganisms in the rhizosphere) holds great potential in the remediation of contaminated soil (Kuiper et al. 2004) In the "rhizosphere effect" plants provide nutrients in the form of root exudates, oxygen, and favorable redox conditions to soil microorganisms, and this in turn results in increased bacterial diversity, population density, and activity compared with bulk soil (Molina et al. 2000; Vilchez et al. 2000; Espinosa-Urgel and Ramos 2001). Root exudates secreted by the roots of Chilli, Corn, and Coriander can increase lindane degradation efficiency of *Klebshiella* sp., *Pseudomonas* sp., and *Pseudoarthrobacter* sp. up to ~10–15 % (Nagpal and Paknikar 2006). Boltner et al. (2007)

used a two-step enrichment approach to isolate five (DS-204B, OF-178A, GOF-203, Ans-PL0, and Ans-PL2) root-colonizing HCH-degrading *Sphingomonas* strains. Out of them two HCH-degrading Sphingomonas strains (GOF-203 and Ans-PL0) exhibited high colonization rate and enhance the rhizoremediation rate.

The GM-microbes with modified genetic composition are found to be efficient tools in enhanced agriculture productivity and bioremediation purpose (Singh et al. 2011a, b, c). A bacterial consortium developed from *Flavobacterium, Vibrio,* and *Burkholderia* was reported with the ability to degrade nearly 90 % of the lindane within 72 h of incubation (Afsar et al. 2005). Paknikar et al. (2005) developed an integrated nano-biotechnological process for producing drinking water free from pesticide residues. FeS-nanoparticles were synthesized by the wet chemical method and were stabilized using a polymer from the fungus *Itajahia* sp. belonging to basidiomycetes. The stabilized FeS-nanoparticles could degrade lindane (5 mg/L) with an efficiency of 94 % in 8 h. Nagpal and Paknikar (2006) isolated 3 bacteria from lindane-contaminated site by enrichment culture technique, i.e., *Klebsiella* sp., *Pseudomonas* sp., *Pseudo-arthrobacter* sp. *Klebsiella* and *Pseudoarthrobacter* having the ability to degrade lindane about 90–92 % in 4 days in association with protozoa by producing 1,2,4-trichlorobenzene as by-product. So, based on this report, it may be deduced that protozoan could be very potential bioagents that may be used in bio-agumentation tool for lindane degradation.

Mertens et al. (2007) use nano-materials in association with *Shewanella oneidensis* as biocatalytic dechlorination of lindane. Abhilash and Singh (2008) employed sugarcane bagasse for bio-treatment of soil containing 50 mg of lindane kg^{-1} soil. They reported that sugarcane bagasse can accelerate lindane degradation by enhanced microbial activity and prevent pesticide mobility through soil column by adsorption. Based on this report it seems that the sugarcane bagasse could be useful as cheaper, easy available alternative for the biostimulation of lindane-impacted soil.

Zhang et al. (2010) developed an autofluorescent *Pseudomonas nitroreducens* with dehydrochlorinase activity for efficient mineralization of lindane. They reported that recombinant strain could rapidly degrade 10 μg/mL lindane in 28 h. Saez et al. (2012) used immobilization technique for lindane removal with four *Streptomyces* strains i.e., A2, A5, A11, and M7. Lindane removal by these immobilized cells was significantly higher than the free cells. Specifically, immobilized cells in cloth sachets showed an improvement of around 25 % in lindane removal compared to the control. Yang et al. (2013) construct an autofluorescent whole-cell biocatalyst degraded lindane completely within 15 days when inoculated with the engineered *S. japonicum* UT26 and the strain could be easily monitored by fluorescence during bioremediation. Singh et al. (2013a, b, c) studied the effect of an integrated nano-biotechnique involving the use of stabilized Pd/Fe bimetallic nanoparticles with *Sphingomonas* sp. strain NM05 in the degradation of lindane. They reported that lindane degradation efficiency is ~1.7–2.1 times greater in integrated system as compared to system containing either NM05 or CMC-Pd/Fe alone. Chaurasia et al. (2013) designed a phototrophic *Anabaena* for bioremediation of traces of lindane prevalent in paddy fields. Salam and Das (2013) reported lindane degradation by bio-micro-emulsions. An embedded bio-nano hybrid system using

nanoscale zinc oxide (n-ZnO) and lindane-degrading yeast *Candida* VITJzN04 has been reported for dechlorination of lindane (Salam et al. 2014).

Lan et al. (2014) constructed a genetically modified microorganism (GMM) named UT26XEGM by introducing a parathion hydrolase gene into an initially lindane-degrading bacterium *S. paucimobilis* UT26. The recombinant bacteria were successfully applied to the bioremediation of lindane. Aresta et al. (2014) isolate bacteria from sponge *Hymeniacidon perlevis* and in vitro investigated ability of both sponge and isolated bacteria to decontaminate lindane-polluted seawater. Sponges showed low mortality in experimental conditions (lindane concentration 1 μg/L) and were able to remove about 50 % of the lindane content from seawater in 48 h. Bacteria removed up to 97 % of lindane after 8 h. 1,3,4,5,6-pentachlorocyc lohexene was produced as metabolite.

A comparative study on lindane remediation potential of four rhizospheric bacterial species, viz. *Kocuria rhizophila, Microbacterium resistens, Staphylococcus equorum,* and *S. cohnii*, was reported by Abhilash et al. (2011). Abhilash et al. (2009b) tested the combined rhizoremediation potential of *Staphylococcus cohnii* subsp. *urealyticus* in association with *Withania somnifera* grown at lindane-spiked soil and concluded that integrated use of rhizospheric-microbial interactions enhanced the dissipation of lindane. Alvarez et al. (2012) studied the dissipation of lindane by native *Streptomyces* strains in the presence of root exudates of *Zea mays* and observed an enhanced dissipation of lindane by the microbes when grown on the root exudates. Becerra-Castro et al. (2013) improved the performance of leguminous shrub *Cytisus striatus* on substrates contaminated with HCH isomers using microbial inoculants. The endophytes *Rhodococcus erythropolis* ET54b and *Sphingomonas* sp. D4 when inoculated to *C. striatus* singly or in combination showed better lindane degradation potential. Kurashvili et al. (2014) demonstrated that chickling vetch (*Lathyrus sativum*), soybean (*Glycine max*), maize (*Zea mays*), alfalfa (*Medicago sativa),* chickpea *(Cicer arietinum),* and lettuce *(Lactuca sativa)* degrade lindane in association with *Pseudomonas* strains. Thus, plant-microbe associations could be a better option for removal of lindane from the contaminated soils and need further study with reference to find out more efficient such association to get rid off from lindane pollution.

11.5 Can Methanotrophs Help in Lindane Degradation?

Methanotrophs, unique group of bacteria, are cosmopolitan and playing major role not in the global carbon and methane (CH_4) cycle but also useful for the biodegradation of hazardous chemicals (Singh et al. 2011a, b, c; Pandey et al. 2014). All aerobic methanotrophs employ the broad substrate enzyme CH_4 monooxygenase (MMO) consuming CH_4. Both forms of MMOs, i.e., particulate CH_4-monooxygenase (pMMO) and soluble CH_4-monooxygenase (sMMO), have been shown to oxidize a range of pollutants, particularly halogenated hydrocarbons (Semrau et al. 2010). Given the ubiquity of aerobic methanotrophs, these microorganisms have been

extensively used for diverse pollutant degradation and methane oxidation (Singh and Singh 2012; Singh and Singh 2013; Singh 2016; Singh and Strong 2016). Many compounds including halogenated alkanes, alkenes, and aromatic compounds have been shown to be degraded by aerobic methanotrophs (Semrau 2011; Tiwari et al. 2015; Singh and Gupta 2016). Methanotrophs species having sMMO degrade greater type contaminants than the pMMO-expressing cells (Burrows et al. 1984). The *Methylocystis* community, an important agent of the methanotrophic population, can degrade halogenated hydrocarbons such as trichloroethylene (TCE) in a tropical soil (Oldenhuis et al. 1989). If MMOs of methanotrophs have broad substrate enzymatic activity and these microbes are ubiquitous in distribution, they can also degrade the various forms of lindane residues in the soils. Though, there are reports that many microbes can degrade diverse pollutants but reports on lindane degradation by methanotrophic bacteria from soils are almost lacking to date. Therefore, there is need to isolate and identify the methanotrophs from lindane-contaminated sites and their role in lindane degradation. Further, research on methanotrophs from lindane-contaminated soils with different concentration of lindane and other chlorinated hydrocarbons will clarify the actual diversity of methanotrophs using the functional genes involved in the degradation of such complex and persistent pollutants. It is also not known that how many types (type-I or type-II) of methanotrophs are present in lindane-contaminated sites. Therefore, our future research work will be focused on the diversity of methanotrophs from lindane-contaminated sites and potential role of these bioagents in lindane remediation.

11.6 Conclusions

Several soil microorganisms capable of degrading and utilizing lindane as carbon and energy source have been reported. In selected bacterial strains, the genes encoding the enzymes involved in the initial degradation of lindane have been cloned, sequenced, and expressed, and the gene products are characterized. More research is needed to understand the basic mechanism of interactions of lindane-degrading microorganisms with the soil environment which regulate the lindane remediation.

In many cases, although the added microorganisms have the ability to degrade the target pollutants, bioremediation does not work successfully in the field conditions. This has been attributed to wide variations in the temperature, pH and moisture-content and other environmental conditions. Limited availability of nutrients, amount of pollutant and its age presence of inhibitory substances and competition with indigenous microflora are the determinants in the lindane degradation. Also the compounds which were biodegraded in the laboratory were present in relatively high concentrations in situ. Thus, the degradation potential observed under laboratory conditions should be studied further under in situ conditions to assess the success of a bioremediation. There is a need of large-scale, more in-depth evaluation of bioremediation protocols using soil with high lindane concentrations. In contrast to using nano-biotechnology, bioremediation is definitely effective and

efficient but the use of nanoparticles may cause an unknown health risk. Therefore, the removal of nanoparticles after the process may be necessary.

After the above discussion it may be deduced that microbes including bacteria, fungi, and methanotrophs are the potential microbial agents that may be exploited for removal and decontamination of lindane-polluted sites. There is need to isolate and identify novel bacterial strains from lindane-contaminated sites having the ability to degrade the lindane at higher rate and their use in decontamination of lindane dump sites. The plant-microbe interactions could be also a better option for removal of lindane resides from the contaminated soils and need further study with reference to find out more efficient plant-microbes interactions to remove the lindane from soil and other polluted natural and agroecosystems.

Acknowledgments We thank Head, Dept. of Environ. Microbiol, BBAU for providing facilities and encouragements. Siddharth Boudh is thankful to UGC, New Delhi for awarding Rajiv Gandhi National Fellowship (Award Letter No: F.1-17.1/2013-14/RGNF-2013-14-SC-UTT-37387).

References

Abhilash PC, Singh N (2008) Influence of the application of sugarcane bagasse on lindane (γ-HCH) mobility through soil column: implication for biotreatment. Bioresour Technol 99:8961–8966

Abhilash PC, Singh N (2009a) Seasonal variation of HCH isomers in open soil and plant-rhizospheric soil system of a contaminated environment. Environ Sci Pollut Res Int 16:727–740

Abhilash PC, Singh N (2009b) *Withania somnifera* dunal-mediated dissipation of Lindane from simulated soil: implications for rhizoremediation of contaminated soils. J Soils Sediments 10:272–282

Abhilash PC, Srivastava S, Singh N (2011) Comparative bioremediation potential of four rhizospheric microbial species against lindane. Chemosphere 82:56–63

Abou-Arab AAK (2002) Degradation of organochlorine pesticides by meat starter in liquid media and fermented sausage. Food Chem Toxicol 40(1):33–41

ACAT and PANNA (2008) Alaska Community Action on Toxics (ACAT) and Pesticide Action Network North America (PANNA) Annex F Submission for Lindane to the POPs Reviewing Committee

Afsar M, Radha S, Girish K, Manonmani HK, Kunhi AAM (2005) Optimization of carbon sources for the preparation of inoculum of hexachlorocyclohexane—degrading microbial consortium. J Food Sci Technol 42:209–213

Alexander M (1999) Biodegradation and bioremediation, 2nd edn. Academic Press, San Diego, pp. 325–354

Alvarez A, Yanez ML, Benimeli CS, Amoroso MJ (2012) Maize plants (*Zea mays*) root exudates enhance lindane removal by native *Streptomyces* strains. Int Biodeterior Biodegrad 66:14–18

Amaraneni SR, Pillala RR (2001) Concentrations of pesticide residues in tissues of fish from Kolleru Lake in India. Environ Toxicol 16:550–556

Anupama KS, Paul S (2010) *Ex situ* and *in situ* biodegradation of lindane by *Azotobacter chroococcu.* J Environ Sci Health A 4558–4566

Aresta A, Nonnis Marzano C, Lopane C, Corriero G, Longo C, Zambonin C, Stabili L (2014) Analytical investigations on the lindane bioremediation capability of the demosponge *Hymeniacidon perlevis*. Mar Pollut Bull 90(1–2):143–149

Arisoy M, Kolankaya N (1997) Biodegradation of lindane by *Pleurotus sajor-caju* and toxic effects of lindane and its metabolites on mice. Bull Environ Contam Toxicol 59:352–359

ATSDR (1999) Toxicological profile for alpha-, beta-, gamma- and delta hexachlorocyclohexane. Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta

Aulakh RS, Gill JPS, Bedi JS, Sharma JK, Joia BS, Ockerman HW (2006) Organochlorine pesticide residues in poultry feed, chicken muscle and eggs at a poultry farm in Punjab, India. J Sci Food Agric 86:741–744

Badea LS, Vogt C, Weber S, Florinte Danet A, Hermann-Richnow H (2009) Stable isotope fractionation of γ-hexachlorocyclohexane (Lindane) during reductive dechlorination by two strains of sulfate-reducing bacteria. Environ Sci Technol 43:3155–3161

Bala K, Sharma P, Lal R (2009) *Sphingobium quisquiliarum* sp nov, a hexachlorocyclohexane (HCH) degrading bacterium isolated from HCH contaminated soil. Int J Syst Evol Microbiol 60(2):429–433

Battu RS, Singh B, Kang BK (2004) Contamination of liquid milk and butter with pesticide residues in the Ludhiana district of Punjab state, India. Ecotoxicol Environ Saf 59(3):324–331

Becerra-Castro C, Kidd PS, Rodriguez-Garrido B, Monterroso C, Santos-Ucha P, Prieto-Fernandez A (2013) Phytoremediation of hexachlorocyclohexane (HCH)-contaminated soils using Cytisus striatus and bacterial inoculants in soils with distinct organic matter content. Environ Pollut 178:202–210

Benimeli CS, Amoroso MJ, Chaile AP, Castro GR (2003) Isolation of four aquatic *Streptomyces* strains capable of growth on organochlorine pesticides. Bioresour Technol 89:133–138

Benimeli CS, Castro GR, Chaile AP, Amoroso MJ (2006) Lindane removal induction by *Streptomyces* sp M7. J Basic Microbiol 46(5):348–357

Benimeli CS, Castro GR, Chaile AP, Amoroso MJ (2007) Lindane uptake and degradation by aquatic *Streptomyces* sp strain M7. Int Biodeterior Biodegrad 59:148–155

Benimeli CS, Fuentes MS, Abate CM, Amarosoa MJ (2008) Bioremediation of Lindane contaminated soil by *Streptomyces* sp M7 and its effects on *Zea mays* growth. Int Biodeterior Biodegrad 61:233–239

Bhanti M, Taneja A (2004) Monitoring of organochlorine pesticide residues in summer and winter vegetables from Agra, India—a case study. Environ Monit Assess 110:341–346

Boltner D, Moreno-Morillas S, Ramos JL (2005) 16S rDNA phylogeny and distribution of *lin* genes in novel hexachlorocyclohexane degrading *Sphingomonas* strains. Environ Microbiol 7:1329–1339

Boyle AW, Haggblom MM, Young LY (1999) Dehalogenation of lindane (gamma-hexachlorocyclohexane) by anaerobic bacteria from marine sediments and by sulfate-reducing bacteria. FEMS Microbiol Ecol 29:379–387

Bueno M, Fillat MF, Strasser RJ, Maldonado-Rodriguez R, Marina N, Smienk H, Gomez-Moreno C, Barja F (2004) Effects of lindane on the photosynthetic apparatus of the cyanobacterium Anabaena: fluorescence induction studies and immunolocalization of ferredoxin-NADP+ reductase. Environ Sci Pollut Res Int 11(2):98–106

Bumpus JA, Tien M, Wright D, Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. Science 228:1434–1436

Burrows KJ, Cornish A, Scott D, Higgins IJ (1984) Substrate specificities of the soluble and particulate methane monooxygenases of *Methylosinus trichosporium* OB3b. J Gen Microbiol 5:335–342

Ceremonie H, Boubakri H, Mavingui P, Simonet P, Vogel TM (2006) Plasmid-encoded γ-hexachlorocyclohexane degradation genes and insertion sequences in *Sphingobium francense* (*Sphingomonas paucimobilis* Sp+). FEMS Microbiol Ecol 257:243–252

Chaudhary P, Kumar M, Khangarot BS, Kumar A (2006) Degradation and detoxification of hexachlorocyclohexane isomers by *Pseudomonas aeruginosa* ITRC-5. Int Biodeterior Biodegrad 57:107–113

Chaurasia AK, Adhya TK, Apte SK (2013) Engineering bacteria for bioremediation of persistent organochlorine pesticide lindane (γ-hexachlorocyclohexane). Bioresour Technol 149:439–445

Choudhary A, Sharma DC (2008) Pesticide residues in honey samples from Himachal Pradesh (India). Bull Environ Contam Toxicol 80:417–422

Costas E, Carrillo E, Ferrero LM, Agrelo M, Garcia-Villada L, Juste J, Lopez-Rodas V (2001) Mutation of algae from sensitivity to resistance against environmental selective agents: the ecological genetics of *Dictyosphaerium chlorelloides* (Chlorophyceae) under lethal doses of 3-(3,4-dichlorophenyl)-1,1-dimethylurea herbicide. Phycologia 40:391–398

Costas E, Flores-Moya A, Perdigones N, Maneiro E, Blanco JL, Garcia ME, Lopez-Rodas V (2007) How eukaryotic algae can adapt to Spain's Rio Tinto: a neo-Darwinian proposal for rapid adaptation to an extremely hostile ecosystem. New Phytol 175:334–339

Costas E, Flores-Moya A, Lopez-Rodas V (2008) Rapid adaptation of algae to extreme environments (geothermal waters) by single mutation allows Noah's Arks for photosynthesizers during the Neoproterozoic snowball Earth? New Phytol 189:922–932

Crawford RL, Crawford DL (1996) Bioremediation: principles and application. Cambridge University Press, Cambridge

Crop Life (2006) Information submitted by CropLife International on behalf of Chemtura Annex E information Stockholm Convention

CSE Report (2003) Analysis of pesticide residues in bottled water (Delhi region)

Cuozzo SA, Rollán GG, Abate CM, Amoroso MJ (2009) Specific dechlorinase activity in lindane degradation by *Streptomyces* sp M7. World J Microbiol Biotechnol 25:1539–1546

Dadhwal M, Jit S, Kumari H, Lal R (2009) *Sphingobium chinhatense* sp nov, a hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. Int J Syst Evol Microbiol 59:3140–3144

Datta J, Maiti AK, Modak DP, Chakrabartty PK, Bhattacharyya P, Ray PK (2000) Metabolism of γ-hexachlorocyclohexane by *Arthrobacter citreus* strain BI-100: identification of metabolites. J Gen Appl Microbiol 46:59–67

De Paolis MR, Lippi D, Guerriero E, Polcaro CM, Donati E (2013) Biodegradation of α-, β-, and γ-hexachlorocyclohexane by *Arthrobacter fluorescens* and *Arthrobacter giacomelloi*. Appl Biochem Biotechnol 170(3):514–524

De Schrijver A, De Mot R (1999) Degradation of pesticides by actinomycetes. Crit Rev Microbiol 25:85–119

Dhananjayan V, Ravichandran B, Rajmohan HR (2012) Organochlorine pesticide residues in blood samples of agriculture and sheep wool workers in Bangalore (Rural) India. Bull Environ Contam Toxicol 89:197–201

Dogra C, Raina V, Pal R, Suar M, Lal S, Gartemann KH, van der Meer JR, Lal R (2004) Organization of lin genes and IS*6100* among different strains of hexachlorocyclohexane-degrading *Sphingomonas paucimobilis*: evidence for horizontal gene transfer. J Bacteriol 186:2225–2235

Dritsa V, Rigas F, Doulia D, Avramides EJ, Hatzianestis I (2009) Optimization of culture conditions for the biodegradation of lindane by the polypore fungus *Ganoderma austral*. Water Air Soil Pollut 204:19–27

Dwivedi V, Niharika N, Lal R (2012) *Pontibacter lucknowensis* sp nov, isolated from a hexachlorocyclohexane dump site. Int J Syst Evol Microbiol 63:309–313

El-Bestawy EA, Abd El-Salam AZ, Mansyc AH (2007) Potential use of environmental cyanobacterial sp in bioremediation of lindane-contaminated effluents. Int Biodeterior Biodegrad 59:180–192

Endo R, Kamakura M, Miyauchi K, Fukuda M, Ohtsubo Y, Tsuda M, Nagata Y (2005) Identification and characterization of genes involved in the downstream degradation pathway of γ-hexachlorocyclohexane in *Sphingomonas paucimobilis* UT26. J Bacteriol 187:847–853

Espinosa-urgel M, Ramos J (2001) Expression of a *Pseudomonas putida* aminotransferase involved in lysine catabolism is induced in the Rhizosphere. Appl Environ Microbiol 67(11):5219–5224

Flores-Moya A, Costas E, Bañares-España E, García-Villada L, Altamirano M, López-Rodas V (2005) Adaptation of *Spirogyra insignis* (Chlorophyta) to an extreme natural environment (sulphureous waters) through pre-selective mutations. New Phytol 166:655–661

Francis AJ, Spanggord RJ, Ouchi GI (1975) Degradation of lindane by *Escherichia coli*. Appl Microbiol 29:567–568

Fuentes MS, Benimeli CS, Cuozzo SA, Amoroso MJ (2010a) Isolation of pesticide-degrading actinomycetes from a contaminated site: bacterial growth, removal and dechlorination of organochlorine pesticides. Int Biodeterior Biodegrad 64:434–441

Fuentes MS, Benimeli CS, Cuozzo SA, Saez JM, Amoroso MJ (2010b) Microorganisms capable to degrade organochlorine pesticides. Microb Biotechnol:1255–1264

Fuentes MS, Saez JM, Benimeli CS, Amoroso MJ (2011) Lindane biodegradation by defined consortia of indigenous *Streptomyces* strains. Water Air Soil Pollut 222:217–231

Garcia-Villada L, Rico M, Altamirano M, Sánchez L, Lopez-Rodas V, Costas E (2004) Occurrence of copper resistant mutants in the toxic cyanobacteria *Microcystis aeruginosa*: characterization and future implications in the use of copper sulphate as algaecide. Water Res 38:2207–2213

González R, García-Balboa C, Rouco M, Lopez-Rodas V, Costas E (2012) Adaptation of microalgae to lindane: a new approach for bioremediation. Aquat Toxicol 109:25–32

Gupta A, Kaushik CP, Kaushik A (2000) Degradation of hexachlorocyclohexane (HCH; α-, β-, γ- and δ-) by *Bacillus circulans* and *Bacillus brevis* isolated from soil contaminated with HCH. Soil Biol Biochem 32:1803–1805

Heritage AD, Mac Rae IC (1977) Identification of intermediates formed during the degradation of hexachlorocyclohexanes by *Clostridium sphenoides*. Appl Environ Microbiol 33:1295–1297

Imai R, Nagata Y, Senoo K, Wada H, Fukuda M, Tagagi M, Yano A (1989) Dehydrochlorination of γ-hexachlorocyclohexane (γBHC) by γ-BHC assimilating *Pseudomonas paucimobilis*. Agric Biol Chem 53:2015–2017

Imai R, Nagata Y, Fukuda M, Takagi M, Yano K (1991) Molecular cloning of a Pseudomonas paucimobilis gene encoding a 17-kilodalton polypeptide that eliminates HCl molecules from γ-hexachlorocyclohexane. J Bacteriol 173:6811–6819

Jagnow G, Haider K, Ellwardt PC (1977) Anaerobic dechlorination and degradation of hexachlorocyclohexane isomers anaerobic and facultative anaerobic bacteria. Arch Microbiol 115:285–292

Johri A, Dua KM, Tuteja D, Saxena R, Saxena DM, Lal R (1998) Degradation of α-, β-, γ- and δ -hexachlorocyclohexanes by *Sphingomonas paucimobilis*. Biotechnol Lett 20:885–887

Kalra RL, Singh B, Battu RS (2003) Organochlorine pesticide residues in human milk in Punjab, India. Environ Pollut 85:47–151

Kaur J, Moskalikova H, Niharika N, Sedlackova M, Hampl A, Damborsky J, Prokop Z, Lal R (2012) *Sphingobium baderi* sp nov, isolated from a hexachlorocyclohexane dump site. Int J Syst Evol Microbiol 63(2):673–678

Kaushik CP, Sharma HR, Jain S, Dawra J, Kaushik A (2007) Pesticide residues in river Yamuna and its canals in Haryana and Delhi, India. Environ Monit Assess 144:329–340

Kobayashi H, Rittmann BE (1982) Microbial removal of hazardous organic compounds. Environ Sci Technol 16:170A–183A

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant-Microbe Interact 17:6–15

Kumar M, Chaudhary P, Dwivedi M, Kumar R, Paul D, Jain RK, Garg SK, Kumar A (2005) Enhanced biodegradation of β and δ -hexachlorocyclohexane in the presence of α- and γ-isomers in contaminated soils. Environ Sci Technol 39:4005–4011

Kumari H, Gupta SK, Jindal S, Katoch P, Lal R (2009) *Sphingobium lactosutens* sp nov, isolated from a hexachlorocyclohexane dump site and *Sphingobium abikonense* sp nov, isolated from oil-contaminated soil. Int J Syst Evol Microbiol 59(9):2291–2296

Kumari K, Sharma P, Tyagi K, Lal R (2010) *Pseudoxanthomonas indica* sp nov, isolated from a hexachlorocyclohexane dumpsite. Int J Syst Evol Microbiol 61(9):2107–2111

Kurashvili M, Adamia G, Ananiashvili T, Amiranasvili L, Varazi T, Pruidze M, Gordeziani M, Khatisashvili G (2014) Plants and microorganisms for phytoremediation of soils polluted with organochlorine pesticides. Int J Biol Veter Agri Food Eng 8(4)

Kuritz T, Wolk CP (1995) Use of filamentous cyanobacteria for biodegradation of organic pollutants. Appl Environ Microbiol 61:234–238

Kuritz T, Bocanera LV, Rivera NS (1997) Dechlorination of Lindane by the cyanobacterium *Anabaena* sp Strain PCC7120 depends on the function of the nir Operon. J Bacteriol 179(10):3368–3370

Lan WS, Lu TK, Qin ZF, Shi XJ, Wang JJ, Hu YF, Chen B, Zhu YH, Liu Z (2014) Genetically modified microorganism *Spingomonas paucimobilis* UT26 for simultaneously degradation of methyl-parathion and γ-hexachlorocyclohexane. Ecotoxicology 23(5):840–850

Lata P, Lal D, Lal R (2011) *Flavobacterium ummariense* sp nov, isolated from hexachlorocyclohexane-contaminated soil, and emended description of *Flavobacterium ceti*. Int J Syst Evol Microbiol 62(11):2674–2679

Lodha B, Bhat P, Kumar MS, Vaidya AN, Mudliar S, Killedar DJ, Chakrabarti T (2007) Bioisomerization kinetics of γ -HCH and biokinetics of *Pseudomonas aeruginosa* degrading technical HCH. Biochem Eng J 35:12–19

Lopez-Rodas V, Agrelo M, Carrillo E, Ferrero LM, Larrauri A, Martín-Otero L, Costas E (2001) Resistance of microalgae to modern water contaminants as the result of rare spontaneous mutations. Eur J Phycol 36:179–190

Lopez-Rodas V, Flores-Moya A, Maneiro E, Perdigones N, Marva F, Garcia ME, Costas E (2007) Resistance to glyphosate in the cyanobacterium Microcystis aeruginosa as a result of preselective mutations. Evol Ecol 21:535–547

Lopez-Rodas V, Marva F, Costas E, Flores-Moya A (2008a) Microalgal adaptation in the stressful acidic, metal-rich mine waters from Mynydd Parys (N Wales, UK) could be due to selection of preselective mutants. Environ Exp Bot 61:43–48

MacRae IC, Raghu K, Bautista EM (1969) Anaerobic degradation of the insecticide Lindane by *Clostridium* sp. Nature (London) 221:859–860

Manickam N, Mau M, Schlomann M (2006) Characterization of the novel HCH-degrading strain, Microbacterium sp ITRC1. Appl Microbiol Biotechnol 69(5):580–588

Manickam N, Reddy MK, Saini HS, Shanker R (2008) Isolation of hexachlorocyclohexane-=degrading *Sphingomonas* sp by dehalogenase assay and characterization of genes involved in γ-HCH degradation. J Appl Microbiol 104:952–960

Manonmani HK, Chandrashekaraiah DH, Sreedhar RN, Elcey CD, Kunhi AAA (2000) Isolation and acclimatization of a microbial consortium for improved aerobic degradation of α-hexachlorocyclohexane. J Agric Food Chem 48:4341–4351

Matsumura F, Benzet HJ, Patil KC (1976) Factors affecting microbial metabolism of γ-BHC. J Pestic Sci 1:3–8

McErlean C, Marchant R, Banat IM (2006) An evaluation of soil colonisation potential of selected fungi and their production of ligninolytic enzymes for use in soil bioremediation applications. Antonie Van Leeuwenhoek 90:147–158

Mertens B, Boon N, Verstraete W (2006) Slow-release inoculation allows sustained biodegradation of gamma-hexachlorocyclohexane. Appl Environ Microbiol 72:622–627

Mertens B, Blothe C, Windey K, Windt WD, Verstraete W (2007) Biocatalytic dechlorination of lindane by nano-scale particles of Pd(0) deposited on *Shewanella oneidensis*. Chemosphere 66(1):99–105

Miyauchi K, Adachi Y, Nagata Y, Takagi M (1999) Cloning and sequencing of a novel metacleavage dioxygenase gene whose product is involved in degradation of γ-hexachlorocyclohexane in *Sphingomonas paucimobilis*. J Bacteriol 181:6712–6719

Miyauchi K, Lee HS, Fukuda M, Takagi M, Nagata Y (2002) Cloning and characterization of linR, involved in regulation of downstream pathway for γ-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. Appl Environ Microbiol 68:1803–1807

Molina L, Ramos C, Duque E, Ronchel MC, Garcia JM, Wyke L, Ramos JL (2000) Survival of *Pseudomonas putida* KT2440 in soil and in the rhizosphere of plants under greenhouse and environmental conditions. Soil Biol Biochem 32:315–321

Mougin C, Pericaud C, Dubroca J, Asther M (1997) Enhanced mineralization of lindane in soils supplemented with the white rot basidiomycete *Phanerochaete chrysosporium*. Soil Biol Biochem 29:1321–1324

Nag SK, Raikwar MK (2008) Organochlorine pesticide residues in bovine milk. Bull Environ Contam Toxicol 80:5–9

Nagata Y, Nariya T, Ohtomo R, Fukuda M, Yano K, Takagi M (1993) Cloning and sequencing of a dehalogenase gene encoding an enzyme with hydrolase activity involved in the degradation of γ-hexachlorocyclohexane in *Pseudomonas paucimobilis*. J Bacteriol 175:6403–6410

Nagata Y, Nariya T, Ohtomo R, Fukuda M, Yano K, Takagi M (1994) Cloning and sequencing of a 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase gene involved in the degradation of γ-hexachlorocyclohexane in *Pseudomonas paucimobilis*. J Bacteriol 176:3117–3125

Nagata Y, Miyauchi K, Takagi M (1999) Complete analysis of genes and enzymes for γ-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. J Ind Microbiol Biotechnol 23:380–390

Nagata Y, Prokop Z, Sato Y, Jerabek P, Kumar A, Ohtsubo Y, Tsuda M, Damborsky J (2005) Degradation of β-hexachlorocyclohexane by haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26. Appl Environ Microbiol 71:2183–2185

Nagpal V, Paknikar KM (2006) Integrated biological approach for the enhanced degradation of lindane. Indian J Biotech 5:400–406

Nalin R, Simonet P, Vogel TM, Normand P (1999) *Rhodanobacter lindaniclasticus* gen nov, sp nov, a lindane-degrading bacterium. Int J Syst Bacteriol 49:19–23

Niharika N, Moskalikova H, Kaur J, Khan F, Sedlackova M, Hampl A, Damborsky J, Prokop Z, Lal R (2012a) *Sphingobium czechense* sp nov, isolated from a hexachlorocyclohexane dump site. Int J Syst Evol Microbiol 63(2):23–728

Niharika N, Moskalikova H, Kaur J, Sedlackova M, Hampl A, Damborsky J, Prokop Z, Lal R (2012b) *Novosphingobium barchaimii* sp nov, isolated from hexachlorocyclohexane-contaminated soil. Int J Syst Evol Microbiol 63(2):667–672

Ohisa N, Yamaguchi M (1978) Gamma-BHC degradation accompanied by the growth of *Clostridium rectum* isolated from paddy field soil. Agric Biol Chem 42:1819–1823

Ohisa N, Yamaguchi M, Kurihara N (1980) Lindane degradation by cell-free extracts of *Clostridium rectum*. Arch Microbiol 25:221–225

Okeke BC, Siddique T, Arbestain MC, Frankenberger WT (2002) Biodegradation of γ-hexachlorocyclohexane (lindane) and α-hexachlorocyclohexane in water and a soil slurry by a Pandoraea species. J Agric Food Chem 50:2548–2555

Oldenhuis R, Vink RLJM, Janssen DB, Witholt B (1989) Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. Appl Environ Microbiol 55:2819–2826

Paknikar KM, Nagpal V, Pethkar AV, Rajwade JM (2005) Degradation of lindane from aqueous solutions using iron sulfide nanoparticles stabilized by biopolymers. Sci Technol Adv Mater 6:370–374

Pandey VC, Singh JS, Singh DP, Singh RP (2014) Methanotrophs: promising bacteria for environmental remediation. Int J Environ Sci Technol 11(1):241–250

Pandit GG, Sahu SK (2002) Assessment of risk to public health posed by persistent organochlorine pesticide residues in milk and milk products in Mumbai, India. J Environ Monit 4:182–185

Papadopoulou K, Rigas F, Doulia D (2006) Lindane degradation in soil by *Pleurotus ostreatus*. WSEAS Trans Environ Dev 5:489–496

Pathak R, Suke SG, Ahmed RS, Tripathi AK, Guleria K, Sharma CS, Makhijani SD, Mishra M, Banerjee BD (2008) Endosulfan and other organochlorine pesticide residues in maternal and cord blood in North Indian population. Bull Environ Contam Toxicol 81:216–219

Pesce SF, Wunderlin DA (2004) Biodegradation of lindane by a native bacterial consortium isolated from contaminated river sediment. Int Biodeterior Biodegrad 54:255–260

Quintero JC, Lu-Chau TA, Moreira MT, Feijoo G, Lema JM (2007) Bioremediation of HCH present in soil by white rot fungus *Bjerkandera adusta* in a slurry batch bioreactor. Int Biodeterior Biodegrad 60:319–326

Quintero JC, Moreira MT, Feijoo G, Lema JM (2008) Screening of white rot fungal species for their capacity to degrade lindane and other isomers of hexachlorocyclohexane (HCH). Cien Inv Agr 35:159–167

Raina V, Suar M, Singh A, Prakash O, Dadhwal M, Gupta SK, Dogra C, Lawlor K, Lal S, van der Meer JR, Holliger C, Lal R (2008) Enhanced biodegradation of hexachlorocyclohexane (HCH) in contaminated soils via inoculation with *Sphingobium indicum* B90A. Biodegradation 19:27–40

Ramteke PW, Hans RK (1992) Isolation of hexachlorocyclohexane (HCH) degrading microorganisms from earthworm got. J Environ Sci Health A 27:2113–2122

Ravel J, Amoroso MJ, Colwell RR, Hill RT (1998) Mercury-resistant actinomycetes from Chesapeake Bay. FEMS Microbiol Lett 162:177–184

Rigas F, Dritsa V, Marchant R, Papadopouloua K, Avramides EJ, Hatzianestis I (2005) Biodegradation of lindane by *Pleurotus ostreatus* via central composite design. Environ Int 31:191–196

Rigas F, Papadopoulou K, Dritsa V, Doulia D (2007) Bioremediation of a soil contaminated by Lindane utilizing the fungus *Ganoderma australe* via response surface methodology. J Hazard Mater 140:325–332

Saez JM, Benimeli CS, Amoroso MJ (2012) Lindane removal by pure and mixed cultures of immobilized actinobacteria. Chemosphere 89:982–987

Sagar V, Singh DP (2011) Biodegradation of Lindane pesticide by non white-rots soil fungus *Fusarium* sp. World J Microbiol Biotechnol 27:1747–1754

Sahu SK, Patnaik KK, Sharmila M, Sethunathan N (1990) Degradation of alpha-, beta-, and gamma-hexachlorocyclohexane by a soil bacterium under aerobic conditions. Appl Environ Microbiol 56:3620–3622

Salam JA, Das N (2013) Lindane degradation by *Candida* VITJzN04, a newly isolated yeast strain from contaminated soil: kinetic study, enzyme analysis and biodegradation pathway. World J Microbiol Biotechnol 30(4):1301–1313

Saxena A, Anand S, Dua A, Sangwan N, Khan F, Lal R (2012) Novosphingobium lindaniclasticum sp nov, a hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. Int J Syst Evol Microbiol 63(6):2160–2167

Semrau JD (2011) Bioremediation via methanotrophy: overview of recent findings and suggestions for future research. FEMS Microbiol 2:209

Semrau JD, DiSpirito AA, Yoon S (2010) Methanotrophs and copper. FEMS Microbiol Rev 34:496–531

Senoo K, Wada H (1989) Isolation and identification of an aerobic gamma-HCH decomposing bacterium from soil. Soil Sci Plant Nutr 35:79–87

Shelton DR, Khader S, Karns JS, Pogell BM (1996) Metabolism of twelve herbicides by Streptomyces. Biodegradation 7:129–136

Shukla G, Kumar A, Bhanti M, Joseph PE, Taneja A (2006) Organochlorine pesticide contamination of ground water in the city of Hyderabad. Mar Pollut Ecotoxicol 32:244–247

Singh JS (2016) Microbes play major roles in ecosystem services. Clim Change Environ Sustain 3:163–167

Singh BK, Kuhad RC (1999) Biodegradation of lindane (γ-hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus*. Lett Appl Microbiol 28:238–241

Singh BK, Kuhad RC (2000) Degradation of insecticide lindane (γ-HCH) by white-rot fungi *Cyathus bulleri* and *Phanerochaete sordid*. Pest Manag Sci 56:142–146

Singh JS, Singh DP (2012) Reforestation: a potential approach to mitigate the excess CH_4 build-up. Ecol Manag Restor 13(3):245–248

Singh JS, Singh DP (2013) Impact of anthropogenic disturbances on methanotrophs abundance in dry tropical forest ecosystems, India. Expert Opin Environ Biol 2:1–3

Singh JS, Gupta VK (2016) Degraded land restoration in reinstating CH_4 sink. Front Microbiol 7(923):1–5

Singh JS, Strong PJ (2016) Biologically derived fertilizer: a multifaceted bio-tool in methane mitigation. Ecotoxicol Environ Saf 124:267–276

Singh JS, Abhilash PC, Singh HB, Singh RP, Singh DP (2011a) Genetically engineered bacteria: An emerging tool for environmental remediation and future research perspectives. Gene 480:1–9

Singh JS, Pandey VC, Singh DP (2011b) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353

Singh JS, Singh DP, Dixit S (2011c) Cyanobacteria: an agent of heavy metal removal. In: Maheshwari DK, Dubey RC (eds) Bioremediation of pollutants. IK International Publisher Co., New Delhi, pp 223–243

Singh R, Manickam N, Mudiam MK, Murthy RC, Misra V (2013a) An integrated (nano-bio) technique for degradation of γ-HCH contaminated soil. J Hazard Mater 258–259:35–41
Singh AK, Garg N, Sangwan N, Negi V, Kumar R, Vikram S, Lal R (2013b) *Pontibacter ramchanderi* sp nov, isolated from hexachlorocyclohexane-contaminated pond sediment. Int J Syst Evol Microbiol 63(8):2829–2834
Singh AK, Garg N, Lata P, Kumar R, Negi V, Vikram S, Lal R (2013c) *Pontibacter indicus* sp nov, isolated from hexachlorocyclohexane-contaminated soil. Int J Syst Evol Microbiol 64(1):254–259
Singh JS, Kumar A, Rai AN, Singh DP (2016) Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front Microbiol 7(529):1–19
Tekere M, Ncube I, Read JS, Zvauya R (2001) Biodegradation of the organochlorine pesticide, lindane by a subtropical white rot fungus in batch and packed bed bioreactor system. Environ Technol 23:199–206
Thomas JC, Berger F, Jacquier M, Bernillon D, Baud-Grasset F, Truffaut N, Normand P, Vogel TM, Simonet P (1996) Isolation and characterization of a novel γ-hexachlorocyclohexane degrading bacterium. J Bacteriol 178:6049–6055
Tiwari S, Singh JS, Singh DP (2015) Methanotrophs and CH_4 sink: effect of human activity and ecological perturbations. Clim Change Environ Sustain 3(1):35–50
UNEP (2009) Report of the conference of the parties of the Stockholm convention on persistent organic pollutants on the work of its fourth meeting. UNEP/POPS/COP4/38, 8 May 2009
Van Doesburg W, van Eekert MHA, Middeldorp PJM, Balk M, Schraa G, Stams AJM (2005) Reductive dechlorination of β-hexachlorocyclohexane (β-HCH) by a *Dehalobacter* species in co-culture with a *Sedimentibacter* sp. FEMS Microbiol Ecol 54:87–95
Van Eekert MHA, van Ras NJP, Mentink GH, Rijnaarts HHM, Stams AJM, Field JA, Schraa G (1998) Anaerobic transformation of β-hexachlorocyclohexane by methanogenic granular sludge and soil microflora. Environ Sci Technol 32:3299–3304
Vijgen J, Yi LF, Forter M, Lal R, Weber R (2006) The legacy of lindane and technical HCH production. Organohalogen Compd 68:899–904
Vijgen J, Abhilash PC, Li YF, Lal R, Forter M, Torres J, Singh N, Yunus M, Tian C, Schäffer A, Weber R (2011) HCH as new Stockholm Convention POPs, a global perspective on the management of lindane and its waste isomers. Environ Sci Pollut Res 18:152–162
Vilchez S, Molina L, Ramos C, Ramos JL (2000) Proline catabolism by *Pseudomonas putida*: cloning, characterization and expression of the *put* genes in the presence of root exudates. J Bacteriol 182:91–99
Willett KL, Ulrich EM, Hites RA (1998) Differential toxicity and environmental fate of hexachlorocyclohexane isomers. Environ Sci Technol 32:2197–2207
Yang C, Liu R, Yuan Y, Liu J, Cao X, Qiao C, Song C (2013) Construction of a green fluorescent protein (GFP)-marked multifunctional pesticide-degrading bacterium for simultaneous degradation of organophosphates and γ-hexachlorocyclohexane. J Agric Food Chem 61(6):1328–1334
Zheng G, Selvam A, Wong JWC (2011) Rapid degradation of lindane (γ-hexachlorocyclohexane) at low temperature by *Sphingobium* strains. Int Biodeterior Biodegrad 65:612–618

Chapter 12
Wastewater Impact on Human Health and Microorganism-Mediated Remediation and Treatment Through Technologies

Sumit Kaushal and Jay Shankar Singh

Abstract Wastewater treatment is an essential process of any region, without which waterborne pathogens can spread resulting in diseases and degradation of receiving water bodies. The wastewater discharge effluents are involved in the degradation process from different receiving sources. The two main processes required for the removal of impurities from wastewater are through chemical and biological means, but due to some drawbacks, these treatments are not initialized; therefore, untreated or inadequately treated wastewater can cause eutrophication in receiving sources of water bodies and also create adverse environmental conditions favoring proliferation of waterborne toxin-producing pathogenic cyanobacteria. Microorganisms such as microalgae and cyanobacteria are effective in wastewater treatment process and are considered to be critical factors in overcoming numerous waterborne diseases. All biological-treatment processes take advantage of microorganisms to use wastewater effluents to provide the energy for microbial metabolism and multiplication. The role of the different microbial groups present in the wastewater treatment systems with importance of microorganism are involved in the removal process of nitrogen and phosphorus indicating that biological treatment system is useful in wastewater treatment systems. The adaptation of nanotechnology is a traditional process of engineering that offers new opportunities in technological wastewater treatment processes. Microalgae biomass cultivation offers an interesting step for wastewater treatments, because tertiary biotreatment, coupled with the production of potentially valuable biomass used for biofuel and bioactive compound productions, helps to minimize the risks to public health and environment. The chapter objective is to review health impacts of wastewater effluents and current advances

S. Kaushal
Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Raibarelly Road, Lucknow 226025, India

J.S. Singh (✉)
Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@bbau.ac.in

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_12

in wastewater treatment due to application of microbes and biotechnological advances. The engineered environmental system with the microbial diversity and their interaction has increased the efficiency of wastewater treatment process.

Keywords Wastewater treatment • Environment • Health impact • Microbes • Microalgae

12.1 Introduction

Wastewater has been adversely affected by anthropogenic influences and combinations of domestic, industrial, commercial, or agriculture activities; surface runoff or storm water and sewer inflow are the possible sources of wastewater (Tilley and Zurbrügg 2014). Wastewater drastically reduces the quality of natural water resources. Cities worldwide generate huge amounts of wastewaters that require recycling treatment and disposal. The recent treatment methods concern over treatment costs, its environmental impact, and the loss of a potentially valuable natural resources. Wastewater reclamation and its utility are viewed as an economically and socially viable enterprise. It is observed that 38,354 million liters per day (MLD) sewage is generated in major cities of India, but the sewage treatment capacity is around 11,786 MLD. The wastewater is deteriorating water quality which is resulting to health problems in the form of waterborne diseases. It is estimated that the projected wastewater from urban areas might cross 120,000 MLD by 2051 and rural areas will generate 50,000 MLD. Currently 884 million and an additional 2.5 billion people lack water sources and sanitation facilities (WHO 2012) despite the remarkable global progress to improve access to drinking water facilities. According to Millennium Development Goals, the access to potable water is increasing but alongside depletion of existing water resources continues as a major concern with projections that approximately 605 million people lack access to safe drinking water by 2015 (UNICEF and World Health Organization 2012). The major increased use of wastewater is for various recreational, agricultural, and aquaculture activities (WHO 2011). The latest estimate resulted that around 22,900 MLD of wastewater generated in the country and only about 5900 MLD (26 %) is treated, while around 17,000 MLD is untreated.

The various microbial populations are found in wastewater treatment, and the presence of such organisms causes waterborne diseases (Akpor and Muchie 2010). The wide variety of viruses, bacteria, and protozoa are present into drinking water supplies or receiving water bodies (Kris 2007). The quantity and diversity of microbes differentiates depending on the intensity and prevalence of infection in the sewered regions. The detection, isolation, and identification of the diversified hazardous microbes in wastewater are always difficult, expensive, and time consuming. Therefore, an indicator organism determines risk of the possible pathogen in wastewater (Paillard and Quentine 2005). Bacteria and algae are considered as common

microbial pollutants in wastewater and lead to various infections and diseases such as diarrhea, dysentery, skin and tissue infections, etc. The two chemical pollutants found in wastewater are nitrogen and phosphorus, acting as limiting nutrients in eutrophication, while there are other chemical pollutants as well such as heavy metals, detergents, and pesticides (Decicco 1979; Larsdotter 2006). Physical, chemical, and biological processes are involved to remove these contaminants and produce safe drinking water sources. The processes for the removal of contaminants in wastewater can be two means such as chemical and biological. Chemical removal is a method of wastewater treatment in which chemicals are added to form particles which settle and remove contaminants. The most common techniques in chemical treatment are coagulation/flocculation, chlorination, chloramination, ozonation, and ultraviolet light (UV) (Joseph and Edwards 1995; Gray 2002). All biological processes have the ability to use microorganisms for wastewater treatment to provide the energy for microbial metabolism and for cell synthesis (Singh et al. 2011a, b, c).

Waste materials are passed through sewage treatment systems on a regular basis and nutrient removal technique process required for the preservation of natural water resources. Identification of microorganisms requires the isolation of pure cultures and investigation of physiological, biochemical traits. DGGE provides characteristic band patterns for different samples, sample profiling, and retaining the possibility of genetic analysis by sequencing of particular bands. FISH technique identifies microorganisms at desired taxonomical level on the basis of specificity of probe used. Confocal laser-scanning microscope allows visualization of three-dimensional microbial structures (granules, biofilms) for understanding biological wastewater treatment. PCR-based methods (cloning and DGGE) are found to be suitable for identifying the microorganisms from wastewater sources. However, appropriate conventional treatment methods are selected along with innovative technologies for treatment of wastewater. This highlights the importance of adequate wastewater management for protection, supply of safe drinking water, and maintenance of public health. The health risks can arise in public from wastewater microbial pathogens, toxic chemicals, and heavy metals. This review focuses on the health risks derived from the presence of microbial pathogens in wastewater; examines the detection, identification, and enumeration of different microbial pathogens in wastewaters, the health risks associated with microbial pathogens in wastewaters, the treatment methods like biological microbial indicators and molecular methods which are used for the removal of microbial pathogens from wastewater; and finally discusses the mechanism of the various microorganisms useful in wastewater treatment systems.

Global concern is related to the wastewater management due to industrialization, increasing population density, and increasing urbanized societies (EPA 1993; McCasland et al. 2008). The effluents from domestic and industrial constitute sources of water contamination which increases treatment cost considerably and also increase the level of chemical and microbial contaminants to water sources (EPA 1996; Eikelboom and Draaijer 1999; Amir et al. 2004). The prevention of wastewater contamination and protection of public health by safeguarding water sources against the spread of waterborne diseases are the reasons for wastewater treatment.

This is accomplished through the metabolic reactions of microorganisms, acceptable quality of wastewater effluents, recycling of microorganisms, or removal of excess microorganisms (Abraham et al. 1997). In municipal wastewater treatment, water quality concerns biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), suspended solids, nitrate, nitrite and ammonia nitrogen, phosphate, salinity, and range of other nutrients and trace metals (Decicco 1979; Brooks 1996).

The presence of high concentrations of wastewater contaminants above the normal level is considered hazardous for receiving water sources because it leads to eutrophication and health risks in humans and animals (EPA 2000; CDC 2002; Runion 2008). Recently, municipal wastewater treatment plants are adapted as a reliable water resource, and in many countries, wastewater treatment reuse is an essential dimension of water resources planning and implementation. Wastewater treatment plants applied to improve the quality of a wastewater involve physical, chemical, and biological processes in primary, secondary, or tertiary stages. The secondary treatment is usually accomplished by biological processes and removes soluble organic matter and suspended solids as a residue from primary treatment microalgae for wastewater treatment, and several researchers have developed techniques for exploiting the algae's fast growth and nutrient removal capacity. The most important common feature of microalgae is that they have oxygen-evolving photosynthesis and use inorganic nutrients and carbon. Microalgal biomass can be used for hydrogen gas production, bioenergy conversion, and production of pharmaceutical substances. Energy recovery from wastewater is achieved in three viable configurations as shown in Fig. 12.1 which includes anaerobic digestion from

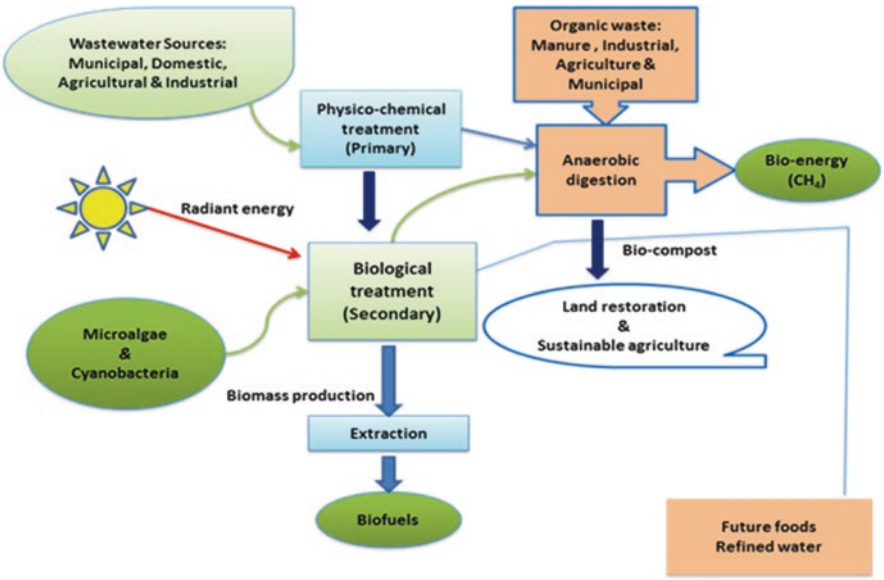


Fig. 12.1 Possible pathways for wastewater treatment and extraction of valuable products

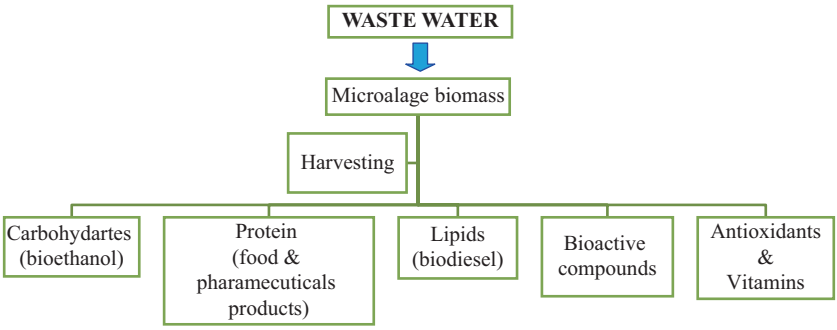


Fig. 12.2 Microalgae biomass in production of useful compounds

primary and secondary treatment to meet treatment energy expenses. Wastewater treatment through bacteria and algae enhances carbon utilization, nutrient removal, and biomass and bioactive production.

Microalgae have great potential in generating energy from biotechnological processes using renewable sources and without compromising food security and agriculture. Depending on the species and growth conditions, microalgae can be selected to produce a wide variety and abundance of lipids, proteins, carbohydrates, and feedstocks important for biofuel and production of nutraceuticals as shown in Fig. 12.2. Consequently, a successful and economically viable microalgae industry producing bioproducts mainly depends on the selection of appropriate microalgae strains.

12.2 Sources of Domestic and Industrial Wastewater

Basically four main types of wastewater are identified as domestic, industrial, agricultural, and urban. Urban wastewater is a combination of domestic, industrial wastewater, surrounding sewage infiltration and rain water, while rural agricultural wastewater consists of farms, agricultural activities, and sometimes contaminated groundwater (Hamdy et al. 2005). Domestic and industrial sewage is a source of contamination. Moreover, agricultural runoff with rich amount inorganic nutrients (P and N) and toxic chemicals may be responsible for surface water eutrophication. Domestic wastewater is sewage which composed of fecal matter (human and animal wastes) together with various wastewater constituents. Such components originate from household activities (washing and bathing) resulting to approximately 32.5 and 67.5 % of domestic sewage (EPA 2013). Initially water used for drinking, food preparation, hot water systems, bathing, personal hygiene, washing, and gardening ultimately form domestic wastewater excreted into the environment (DWAF 1996). Household domestic wastewater contributes to different overall nutrients and comprises the discharged effluent. Industrial wastewater as sewage

consists of pulp, paper, petrochemical runoff, as well as various chemicals, salts, and acids. The composition of industrial wastewater varies based on contaminant and pollutant composition with classification into inorganic and organic industrial wastewater (Rosenwinkel et al. 2005). Therefore, sources differentiate widely in composition and require basic tertiary treatments in order to comply with discharge regulations.

12.3 Composition of Typical Wastewater

Natural water sources receive pollution from different sources, and wastewater composition is a reflection of the technologies practiced in various producing areas (Gray 1989). It is a complex mixture of natural organic, inorganic materials, and man-made compounds. Three quarters of organic carbon in sewage are carbohydrates, fats, proteins, amino acids, and volatile substances. The inorganic constituents include high quantities of sodium, calcium, potassium, magnesium, chlorine, sulfur, phosphate, bicarbonate, ammonium salts, and heavy metals (Tebbutt 1983; Horan 1990; Lim et al. 2010). Different sources of pollutants include discharged raw or treated sewage from towns and villages and from manufacturing or industrial plants, run-off from agricultural land, and leachates from solid waste disposal sites (Horan 1990).

12.4 Microbiological Composition of Wastewater

Wastewater environment is a medium for a wide range of microorganisms, especially harmless bacteria, viruses, and protozoa that are used in biological sewage treatment and pathogenic microorganisms in sewage. Microorganisms cause cholera, typhoid, and tuberculosis; viruses cause infectious hepatitis; protozoa cause dysentery; and the eggs of parasitic worms exist in sewage (Glynn Henery 1989; Shaaban et al. 2004). The efficiency of disinfecting sewage is generally estimated by the extent of removal of total coliform organisms (Sebastian and Nair 1984).

12.5 Characteristics of Wastewater Effluents

12.5.1 Physicochemical Characteristics

The physicochemical characteristics of wastewater include pH; dissolved oxygen (DO) chemical or biological oxygen demand; solids in suspended or dissolved form; nitrogen occurring as nitrite, nitrate, and ammonia; phosphate; and metals

(Decicco 1979; Larsdotter 2006). The hydrogen ion concentration is quality parameter of natural and wastewaters and describes the acid or base properties of wastewater. Wastewater influent in septic conditions has pH <7, while values <5 and pH >10 indicate industrial wastes and noncompatibility with biological operations. The existence of biological life is quite narrow when pH concentration ranges typically from 6 to 9, and indication of extreme pH damages biological processes in biological treatment units (EPA 1996; Gray 2002). Some another parameter shows significant effect on the characteristics of water such as dissolved oxygen as it is required for the respiration of aerobic microorganisms and other life forms. The actual quantity of oxygen present in solution is governed by the solubility, temperature, partial pressure of the atmosphere, and the concentration of impurities such as salinity and suspended solids in the water (EPA 1996; Metcalf and Eddy 2003). Oxygen demand in the form of BOD or COD is used by microorganisms as they feed upon organic solids in wastewater (Gray 2002) and dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter. BOD test widely includes the requirement of a high concentration of active acclimated microorganisms and treatment while dealing with toxic wastes and reduces the effects of nitrifying organisms. Similarly, the COD which measures the oxygen equivalent of the organic material in wastewater is oxidized chemically, and COD is always higher than the BOD because COD measures substances which are both chemically and biologically oxidized.

Heavy metals are referred as persistent pollutants in wastewater and cannot be degraded, but accumulate with the food chain, producing human health risks and causing ecological disturbances. Heavy metals in wastewater are from residential dwellings, groundwater infiltration, and industrial discharges. The accumulation of metals in wastewater depends on many local factors like type of industries in the region, way of life, and awareness of the impact on the environment through the careless hazardous disposal of wastes (Hussein et al. 2005; Silvia et al. 2006). An excess amount of phosphorus in natural waters sources usually leads to eutrophication. Therefore, controlling phosphorus discharge from municipal and industrial wastewater treatment are preventing eutrophication of surface waters (Department of Natural Science 2006).

12.5.2 Microbiological Characteristics

The microorganisms found in wastewater are like viruses, bacteria, fungi, protozoa, and helminthes and various microorganisms contributing to numerous waterborne outbreaks (Kris 2007). Microorganisms are involved in secondary treatment of wastewater for removal of organic matter and while undergoing different treatment processes cause degradation of solids resulting in lesser sludge production (Ward-Paige et al. 2005). Wastewater microbes involved in the process of nutrient recycling like phosphate, nitrogen, and heavy metals and microbial pollutants act as an indicator of water quality. Similarly, detection, isolation, and identification of

different microbial pollutants in wastewater are always expensive and time consuming, and indicator organisms are used to determine relative risk of the availability of certain pathogen in wastewater (Paillard and Quentine 2005). Enteric bacteria, such as coliforms, *Escherichia coli*, and fecal streptococci, are indicators of fecal contamination in water sources (DWAF 1996; Momba and Mfenyana 2005).

12.6 Microbial Pathogens in Wastewater

Microbial pathogens present in wastewater are divided into three separate groups, and these groups are viruses, bacteria, and pathogenic protozoan/helminthes. The pathogens are enteric in origin, excreted in fecal matter that contaminate environment and gain access to new hosts through ingestion. Microbial pathogens are detected in wastewaters, and many microbial pathogens in wastewaters are enteric in origin because of non-enteric illnesses (e.g., *Legionella* spp., *Mycobacterium* spp., and *Leptospira*) (Fliermans 1996; Neumann and Behringer 1997; Wilson and Fujioka 1995). Gastrointestinal infections are among the most common diseases caused by bacterial pathogens in wastewater including diarrhea, cholera, salmonellosis, and dysentery. The contamination of food by water containing toxin-producing organisms such as *Staphylococcus aureus*, *Salmonella* spp., *E. coli*, or *Clostridium perfringens* results in outbreaks of food poisoning. Mycobacterium ulcerans which causes subcutaneous ulcerous lesions on body extremities has been implicated through epidemiological evidence in wastewater and results in infections through contact with the wastewater (Johnson et al. 1996). Many opportunistic pathogens of the natural microbial population have the ability to increase in number on the presence of sufficient nutrients. The wastewaters often have high nutrient loads; high numbers of these opportunistic pathogens can be present, increasing the risk of infections occurring from them.

12.7 Impacts of Wastewater Effluents

The qualities of wastewater effluents are responsible for the degradation of the receiving water bodies, such as lakes, rivers, streams, etc. The deleterious effects of polluted wastewater effluents on the quality of natural water body sources are manifold and depend on discharge, chemical, and microbiological concentration/composition of the effluents. It also depends on the discharge of suspended solids or organic matter or hazardous pollutants like heavy metals and organochlorines and characteristics of the receiving waters (Owuli 2003). Eutrophication of water sources creates environmental conditions that initialize the growth of toxin-producing cyanobacteria, and chronic exposure to such toxins causes gastroenteritis, liver damage, nervous system impairment, skin irritation, and liver cancer in animals (EPA 2000; Eynard et al. 2000; WHO 2006); similarly, recreational water users in contact with the infected water are at health risk (Resource Quality Services 2004).

12.8 Health Impacts

Diseases caused by bacteria, viruses, and protozoa are the health hazards associated with untreated drinking and recreational waters, and main sources of these microbial contaminants in wastewater are human and animal wastes (WHO 1997, 2006; EPA 2000). Microbial pathogens contribute to numerous waterborne outbreaks, and many microbial pathogens in wastewater cause chronic diseases. Microorganisms cause infections, such as diarrhea, dysentery, skin and tissue infections, etc. Similarly, disease-causing bacteria found in water include several types of bacteria, such as *E. coli* O157:H7, *Listeria*, *Salmonella*, Leptospirosis, *Vibrio*, *Campylobacter*, etc. (CDC 1997). The tests of total coliform and fecal coliform nonpathogenic bacteria indicate the presence of pathogenic bacteria (EPA 1996).

Detectable health effects are found at levels of 2300–2400 total coliforms per 100 ml in recreational waters. Nitrogen and phosphorus stimulate the growth of toxic species of phytoplankton in both fresh and marine waters, and consumption of toxic algae or organisms causes serious harm to humans and terrestrial animals. The toxins produced by microscopic algae can reach undesirable concentrations during eutrophication and also lead to the production of algal blooms. Algal blooms are responsible for depletion of dissolved oxygen and cause water quality problems (EPA 2000) and health risks associated with untreated wastewater. Health risk associated with wastewater effluents results from the use of chlorine as a disinfectant in treatment. Although chlorination is effective in the elimination of typhoid fever, cholera, and other waterborne diseases, the oxidizing power of chlorine reacts with naturally occurring organic material in raw wastewater effluent to produce chlorinated compounds (Wigle 1998). Ammonia in aquatic environments even at normal concentrations may lead to several human health impacts such as pulmonary edema (WHO 1997, 2006). Methemoglobinemia is a significant health problem associated with nitrate in water, and blood contains an iron-based compound (hemoglobin) that carries oxygen; therefore, under the presence of nitrite, hemoglobin is converted to methemoglobin as it is unable to carry oxygen.

The environmental impact of untreated wastewater effluent is linked to health and phenomenon of bioaccumulation and biomagnifications of contaminants. The phenomenon of bioaccumulation of certain substances which are present in low concentrations is measurable in water sometimes in high concentrations in tissues of plants and animals. Acute impacts from wastewater effluents are generally because of high levels of ammonia and chlorine, oxygen-demanding materials, toxic concentrations of heavy metals, and organic contaminants. Nutrient-induced production of aquatic plants in receiving water bodies leads to detrimental consequences: (1) algal clumps, odors, and decoloration of the water; (2) dead macrophysics and phytoplankton, stimulating microbial breakdown processes and causing oxygen depletion, resulting in death of desirable aquatic life; and (3) algal blooms submerging aquatic vegetation, eliminating photosynthesis and productivity (Kurosu 2001; McCasland et al. 2008).

12.9 Presence of Microbial Pathogens in Wastewater

It is imperative to determine the presence or absence of microbial pathogens in wastewater used in reclamation projects. The efficient enumeration of microbial pathogens in a wastewater treatment allows an effective risk assessment to be made prior to the recycling of the wastewater. There are a number of established methods for the detection of most microbial pathogens of these methods showing major limitations. These limitations are associated with time taken to isolate and identify pathogen and determine the numbers of pathogens in a sample along with accuracy of detection.

12.10 Physicochemical and Microbiological Indicators

Some microorganisms determine the presence of pathogenic microorganisms and function effectively as indicators for such pathogens, being present in equivalent or higher numbers and more resistant to environmental factors and treatment processes than the pathogenic microorganisms. Microbial pathogens present in waters and wastewaters are fecal in origin, and detection of fecal contamination of water is the aim of water testing authorities. *Bacteroides* is bacterium which is examined for potential use as an indicator and is an obligate anaerobe like the bifidobacteria. The recent development of DNA probes for polymerase chain reaction (PCR) detection alleviates requirement for culturing and uses *Bacteroides* strains as indicators of fecal pollution (Kreader 1995). The quality control of wastewater treatments was monitored using physicochemical and microbiological indicators, and association of treatments with effluents was analyzed. The microbiological indicators monitored heterotrophic plate count (HPC), total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), and sulfite-reducing clostridia (SRC). The wastewater treatment was evaluated through determination of ammonia, biological oxygen demand (BOD), chemical oxygen demand (COD), suspended dissolved and total solids, total nitrogen, pH, and phosphate levels.

12.11 Wastewater Treatment

12.11.1 Biological Wastewater Treatment Systems

The fundamental reason for wastewater treatment is to control the effect of water source pollution and to protect public health by safeguarding water sources against the spread of diseases through a variety of on-site or off-site treatment systems. Therefore, off-site (activated sludge, trickling filters, stabilization ponds,

constructed wetlands, membrane bioreactors) wastewater treatment systems (USEPA 2005) and biological wastewater treatment are divided into two treatment groups, on-site and off-site treatment systems, which require proper maintenance and demand for public health and environmental impacts. Biological wastewater treatment process achieves maximal reduction of biological oxygen demand of wastewater with a minimal reduction of biological solids, and it is accomplished by removing substances which have increase demand for oxygen from the system through the metabolic reactions of the microorganisms, the separation and settling of activated sludge solids to create an acceptable quality of wastewater effluents, and recycling of microorganisms or removal of excess microorganisms from the system (Abraham et al. 1997).

12.11.2 Molecular Techniques for Wastewater Treatment

Identification of microorganisms by conventional methods requires the isolation of pure cultures followed by characterization experiments. Molecular techniques include denaturant gradient gel electrophoresis (DGGE) and fluorescent in situ hybridization (FISH) with DNA probes. DGGE is a rapid and simple method which provides characteristic band patterns for different samples allowing quick sample profiling while retaining thorough genetic analysis by sequencing of particular bands. FISH identifies microorganisms at any desired taxonomical level depending on the specificity of the probe, and combination along with confocal laser-scanning microscope allows visualization of three-dimensional microbial structures (granules, biofilms). PCR-based methods including cloning and DGGE are suitable for identifying the microorganisms forming the sludge. FISH is used for elucidation of the composition, quantification, and distribution of different bacterial groups in granules and biofilms as well as their structure.

FISH of 16S rRNA sequences provides phylogenetic information and distinguishes independently different populations based on activity, and quantitative dot blot hybridization was applied to several population analyses of wastewater treatment systems, with inherent limitations associated with established methods used for detection of various microbial pathogens in wastewaters. PCR is used as the standard method or modified to semi-nested or nested PCR methods (Gajardo et al. 1995; Mayer and Palmer 1996; Straub and Gerba 1995), and detection limits for PCR methods have increased through use of membrane hybridization detection of PCR products with specific DNA probes (Hay et al. 1995; Laberge and Griffiths 1996; Schwab and Sobsey 1995) or by using enzyme-linked immunoassay (ELISA) (Ritzler and Altwegg 1996). Highly probable wastewater samples contain several types of microbial pathogen and multiplex PCR used to detect more than one target in a single PCR reaction (Pepper and Gerba 1997; Picone and Fricker 1997; Rochelle and Wolfe 1997; Way et al. 1993). Several methods detailed above have been used

for the production of commercial kits for the detection of various microorganisms in clinical, food, and environmental samples. The presence of β-galactosidase and β-glucuronidase coliforms and enterococci has been used in the development of several commercial kits for the rapid detection of these organisms in sewage and wastewater samples and examples of kits include ColiPAD® (IDEXX) and ColiTrak Plus (Biocontrol).

12.11.3 Innovative Technologies for Wastewater Treatment

Many technologies facilitate the implementation of systems and improve decentralized and centralized water and resource management. Tools are available for:

1. More efficient capture and local use of storm water to conserve local water resources
2. Improved water conservation for reducing water consumption without compromising standards of living
3. The reclamation of wastewater
4. The management and extraction of energy from wastewater stream
5. The recovery of nutrients
6. The separation of specific wastewater sources:

- *Membrane filtration systems*: Membrane systems critical to development of advanced water reclamation systems and development of improved systems. Immersed ultrafiltration membranes provide excellent pretreatment to remove several dissolved constituents, and development of membrane filtration systems led to development of both advanced water-treatment technology and workhouse of water-reclamation industry. MBRs and biological solids residence times (SRTs) increased biological treatment and retention of pathogens.
- *Nanotechnology*: Nanotechnology based membranes are used for waste water treatments with fewer fouling characteristics (Kim et al. 2008).
- *Microbial fuel cells*: Electrical energy extracted directly from organic matter present in waste stream by using electron transfer to capture energy produced by microorganisms for metabolic processes (Logan and Rabaey 2006). Microorganisms as bio-film have potential to produce electrical energy directly from the waste organic matter.
- *Natural treatment systems*: Characterization of processes in natural treatment systems (NTSs) enabling advantage of natural processes to improve water quality, water reclamation (Kadlec and Knight 1996), and a variety of physical, chemical, and biological processes functions simultaneously to remove contaminants including nutrients, pathogens, and microconstituents.

12.12 Conclusion and Future Prospective

The main reason for treating wastewater is to prevent the spread of diseases by safeguarding water sources against pollution. Treatment of wastewater is one of the strategies for the management of water quality. Our understanding of the microbial community structure in wastewater treatment systems continues to advance rapidly owing to the ongoing development and application of molecular methods. Contaminants like hydrocarbon, heavy metals, nitrogen, and phosphorus in distributed water and discharged wastewater are a constant area of concern because domestic and industrial wastewaters are large sources of effluents discharged into natural water bodies. The quality of wastewater effluents is responsible for the degradation of the receiving water bodies with the impacts of such degradation resulting in the spread of various waterborne diseases, decreased levels of dissolved oxygen, decreased water quality, release of toxic substances, bioaccumulation in aquatic life, and increased nutrient loads. Algae-based system for wastewater treatment relies on efficiency or mechanism of algal cells to effectively assimilate both organic and inorganic carbon and other nutrients such as N, P from wastewater for algal biomass resulting in biodiesel and bio-compound production. In addition, microalgae-based wastewater treatment has advantages such as algal biomass and other bio-products of traditional wastewater treatment process being used as energy-rich source. Moreover, wastewater treatment screens microalgae strains for highly efficient wastewater remediation and maximal algal biomass production.

With the assurance of an effective water quality management, appropriate wastewater treatment strategies are vital and can be achieved through appropriate treatment processes to minimize the risks to public health and environment. Unpolluted wastewater discharge into receiving water bodies needs to be carefully planned for adequate and suitable treatment and regular monitoring enhanced through the utilization of technologies. Adverse effects of pathogenic microorganisms are the major risk associated with the recycling of wastewaters. Methods for the detection of pathogenic and indicator microorganisms are improving, but further research and ratification of new methods is required. Many factors can influence choices of treatment processes including different microbial pathogens present, microbe's resistance to treatment processes, use for intended recycled wastewater, and potential contact with public. The gaps in the knowledge of pathogenic microorganisms in wastewater are a thorough survival and persistence of the different microbial types at different conditions and environments. The rapid development of efficient detection methods particularly PCR lead to more efficient processes.

However, such a method requires quantitative knowledge of all of the factors mentioned above, along with information on pathogen infection rates and health consequences which can efficiently remove risks. Biosensors are based on indicators of microbial water quality technologies and gene recognition in the microarray format for detecting microorganisms. There is a need for novel water technologies to ensure high-quality drinking water, eliminate micropollutants, and use flexibly

adjustable water treatment systems. The adaptation of highly advanced nanotechnology to traditional process offers new opportunities for development of advanced wastewater technology processes. One of the most important advantages of nanotechnology is the ability to integrate various properties resulting in multi-functional systems such as elimination of contaminants and limitations of health risks. The review shows that novel techniques have increased our insight into the vast diversity and interaction of microorganisms with wastewater treatment systems.

References

Abraham PJV, Butter RD, Sigene DC (1997) Seasonal changes in whole-cell metal levels in protozoa of activated sludge. Ecotoxicol Environ Saf 38:272–280

Akpor OB, Muchie M (2010) Bioremediation of polluted wastewater influent phosphorus and nitrogen removal. Sci Res Essays 5(21):3222–3230

Amir HM, Ali RM, Farham K (2004) Nitrogen removal from wastewater in a continuous flow sequencing batch reactor. Pak J Biol Sci 7(11):1880–1883

Brooks PC (1996). Investigation of temperature effects on denitrifying bacterial populations in biological nutrient removal (BNR) system. MS thesis, Virginia Poly Inst and State Univ, Blacksburg, Virginia, USA

CDC (1997) Outbreaks of *Escherichia coli O157:H7* infection associated with eating alfalfa sprouts Michigan and Virginia. Morb Mortal Wkly Rep 46(32):741

CDC (2002) U.S. Toxicity of Heavy Metals and Radionucleotides Department of Health and Human Services, Centers for Disease Control and Prevention. Savannah river-site health effects subcommittee (SRSHES) meeting. www.cdc.gov/nceh/radiation/savannah/. Accessed 7 Dec 2007

Decicco BT (1979) Removal of eutrophic nutrients from wastewater and their bioconversion to bacterial single cell protein for animal feed supplements phase II: WRRC report 15

Department of Natural Science (2006) Wastewater characterization for evaluation of biological phosphorus removal. www.dnr.state.wi.us/org/water. Accessed 13 Jun 2006

DWAF (1996) South African water quality guidelines, vol 1: Domestic water use, 2nd edn. The Department of Water Affairs and Forestry, Pretoria, South Africa. pp 240–256

Eikelboom DH, Draaijer A (1999) Activated sludge information system (ASIS). www.asissludge.com. Accessed 9 Nov 2007

EPA (1993) Constructed wetlands for wastewater treatment and wildlife habitat: EPA832-R-93-005. www.epa.gov/. Accessed 14 Jan 2008

EPA (1996) U.S. Environmental Protection Agency, American Society of Civil Engineers, and American Water Works Association technology transfer handbook: management of water treatment plan residuals EPA/625/R-95/008. Office of Research and Development, Washington, DC, p 281

EPA (2000) Nutrient criteria technical guidance manual-rivers and streams EPA-822-B-00-002. U.S. Environmental Protection Agency, Washington, DC, p 126

EPA (2013) An environmental risk based methodology to assist in the prioritisation of the inspection of domestic waste water treatment systems. Environ Prot Agency, Wexford, p 59

Eynard F, Mez K, Walther JL (2000) Risk of cyanobacteria toxins in Riga waters (LATVIA). Water Res 30(11):2979–2988

Fliermans CB (1996) Ecology of *Legionella*: from data to knowledge with a little wisdom. Microb Ecol 32:203–228

Gajardo R, Pint RM, Bosch A (1995) Polymerase chain reaction amplification and typing of rotavirus in environmental samples. Water Sci Technol 31:371–374

Glynn Henery J (1989) Water pollution. In: Heinke GW, Glynn Henery J (eds) Environmental science and engineering. Prentice-Hall, Englewood Cliffs, NJ, pp 297–329
Gray NF (1989) Biology of wastewater treatment. Oxford University Press, Oxford, pp 47–61
Gray NF (2002) Water technology: an introduction for environmental scientists and engineers. Butterworth-Heinemann, Oxford, pp 35–80
Hamdy A, Shatanawi M, Smadi H (2005) Urban wastewater problems, risks and its potential use for Irrigation the use of non-conventional water resources. Bari: CIHEAM/EU-DG Res, pp 15–44
Hay J, Seal DV, Billcliffe B, Freer JH (1995) Non-culturable *Legionella pneumophila* associated with Acanthamoeba castellanii detection of the bacterium using DNA amplification and hybridization. J Appl Bacteriol 78:61–65
Horan NJ (1990) Biological wastewater treatment systems theory and operation. Wiley, Chichester, UK, p 310
Hussein H, Farag S, Kandil K, Moawad H (2005) Tolerance and uptake of heavy metals by Pseudomonads. Process Biochem 40:955–961
Johnson PDR, Veitch MGK, Leslie DE, Flood PE, Hayman JA (1996) The emergence of Mycobacterium ulcerans infection near Melbourne. Med J Aust 164:76–78
Joseph D, Edwards PE (1995) Industrial wastewater engineering. CRC, Seattle, WA 10(3): 434–442
Kadlec RH, Knight RL (1996) Treatment wetlands. CRC, Boca Raton, FL, p 893
Kreader CA (1995) Design and evaluation of bacteroides DNA probes for the specific detection of human fecal pollution. Appl Environ Microbiol 61:1171–1179
Kris M (2007) Wastewater pollution in China. www.dbc.uci/
Kurosu O (2001) Nitrogen removal from wastewaters in microalgae bacterial-treatment ponds, p 13. http://www.socrates. Accessed 10 Jun 2007
Laberge I, Griffiths MW (1996) Detection of Cryptosporidium parvum in raw milk by PCR and oligonucleotide probe hybridization. Appl Environ Microbiol 62:3259–3264
Larsdotter K (2006) Microalgae for phosphorus removal from wastewater in a Nordic climate. A doctoral thesis from the School of Biotech, Royal Inst of Tech, Stockholm, Sweden, p 39
Lim S, Chu W, Phang S (2010) Use of Chlorella vulgaris for bioremediation of textile. Wastewater J Bioresour Technol 101:7314–7322
Logan BE, Rabaey K (2006) Microbial fuel cells: methodology and technology. Environ Sci Tech 40(17):5181–5192
Mayer CL, Palmer CJ (1996) Evaluation of PCR, nested PCR, and fluorescent antibodies for detection of Giardia and Cryptosporidium species in wastewater. Appl Environ Microbiol 62: 2081–2085
McCasland M, Trautmann N, Porter K, and Wagenet R (2008) Nitrate: health effects in drinking water. pmep.cee.comell.edu. Accessed 5 Apr 2008
Metcalf X, Eddy X (2003) Wastewater engineering: treatment and reuse. In: Wastewater engineering, treatment, disposal and reuse, 4th edn. Tata McGraw Hill, New Delhi, India, p 1819
Momba MNB, Mfenyana C (2005) Inadequate treatment of wastewater: a source of coliform bacteria in receiving surface water bodies in developing countries case study: Eastern Cape Province of South Africa. In: Lehr JH, Keeley J (eds) Water encyclopaedia domestic, municipal and industrial water supply and waste disposal. Wiley, pp 661–667
Neumann MR, Behringer K (1997) Comparison of methods for isolation of mycobacterium from water. Appl Environ Microbiol 63:547–552
Owuli MA (2003) Assessment of impact of sewage effluents on coastal water quality in Hafnarfjordur, Iceland: The United Nations Fishery Training Program, Final Rep. Environ Eng J 4:156–161
Paillard D, Quentine C (2005) Occurrence of Listeria spp. in effluents of French urban wastewater treatment plants. Appl Environ Microbiol 71(11):7562–7566
Pepper IL, Gerba CP (1997) Detection of microorganisms in soils and sludges. In: Toranzos GA (ed) Environmental applications of nucleic acid amplification techniques. Tech Publish Comp, Lancaster, PA, pp 95–111

Picone T, Fricker E (1997) Detection of *Legionella* and *Legionella pneumophila*. In: Environmental water samples using the polymerase chain reaction. Technomic Company, Lancaster, PA, pp 159–182

Resource Quality Services (2004) Eutrophication and toxic cyanobacteria. Department of Water Affairs and Forestry, South Africa, p 25. www.dwaf.gov.za/WQ. Accessed 14 Mar 2007

Ritzler M, Altwegg M (1996) Sensitivity and specificity of a commercially available enzyme-linked immunoassay for the detection of polymerase chain reaction amplified DNA. J Microbiol Methods 27:233–238

Rochelle PA, Wolfe RL (1997) Comparison of primers and optimization of PCR conditions for detection of Cryptosporidium parvum and Giardia lamblia in water. Appl Environ Microbiol 63:106–114

Rosenwinkel KH, Austermann-Haun U, Meyer H (2005) Industrial wastewater sources and treatment strategies. In: Jörd HJ, Wint J (eds) Environmental biotechnology: concepts and applications. Wiley, Germany, pp 49–78

Runion R (2008) Factors to consider in wastewater-2 Ezine Articles (April 14). ezinearticles.com/. Accessed 9 May 2008

Schwab KJ, Sobsey MD (1995) Concentration and purification of beef extract mock eluates from water samples for the detection of enteroviruses, hepatitis A virus, and Norwalk virus by reverse transcription-PCR. Appl Environ Microbiol 61:531–537

Sebastian S, Nair K (1984) Total removal of coliforms and E. coli from domestic sewage by high rate pond mass culture of Scenedesmus obliquus. Environ Pollut 34:197–206

Shaaban A, Haroun BM, Ibraheem IBM (2004) Assessment of impact of Microcystis aeruginosa and Chlorella vulgaris in the uptake of some heavy metals from culture media, pp 433–450

Silvia D, Ana M, Juan CG (2006) Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa. Environ Int 32(6):711–717

Singh JS, Abhilash PC, Singh HB, Singh RP, Singh DP (2011a) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480:1–9

Singh JS, Pandey VC, Singh DP (2011b) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353

Singh JS, Singh DP, Dixit S (2011c) Cyanobacteria: an agent of heavy metal removal. In: Maheshwari DK, Dubey RC (eds) Bioremediation of pollutants. IK International Publisher, New Delhi, pp 223–243

Straub TM, Gerba CP (1995) Comparison of PCR and cell culture for detection of enteroviruses in sludge-amended field soils and determination of their transport. Appl Environ Microbiol 61:2066–2068

Tebbutt THY (1983) Principles of water quality control, 3rd edn. Pergamon Press, Oxford, p 229

Tilley E, Zurbrügg C (2014) Compendium of sanitation systems and technologies, 2nd revised edition. Swiss Federal Inst of Aquatic Sci and Tech (Eawag), Duebendorf, Switzerland, p 175

UNICEF, World Health Organization (2012) Progress on drinking water and sanitation. Joint monitoring programme for water supply and sanitation, UNICEF, New York, p 66

USEPA (2005) Pollution prevention (P2) Framework: EPA-748-B-04-001, p 174

Ward-Paige CA, Risk MJ, Sherwood OA (2005) Reconstruction of nitrogen sources on coral reefs: δ15N and δ13C in gorgonians from Florida Reef Tract. Mar Ecol Prog Ser 296:155–163

WHO (1997) Nitrogen oxides: environmental health criteria, 2nd edn. No. 54. World Health Organization, Geneva, Switzerland, pp 175–199

WHO (2006) Guidelines for the safe use of wastewater, excreta and greywater, vol 3. World Health Organisation Press, Geneva, Switzerland, p 135

WHO (2012) United Nations water global annual assessment of sanitation and drinking water (GLAAS): the challenge of extending and sustaining services. World Health Organisation, Geneva, Switzerland, p 112

Wigle DT (1998) Safe drinking water: a public health challenge. Chronic Dis Can 19(3):1–8

Wilson R, Fujioka R (1995) Development of a method to selectively isolate pathogenic *Leptospira* from environmental samples. Water Sci Technol 31:275–282

World Health Organisation (2011) Guidelines for drinking-water quality, 4th edn. WHO, Geneva, Switzerland, p 564

Index

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3