Handbook of
Hydrocarbon and Lipid Microbiology

SPRINGER
REFERENCE

Terry J. McGenity *Editor*

Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology

Springer

Handbook of Hydrocarbon and Lipid Microbiology

This handbook is the unique and definitive resource of current knowledge on the diverse and multifaceted aspects of microbial interactions with hydrocarbons and lipids, the microbial players, the physiological mechanisms and adaptive strategies underlying microbial life and activities at hydrophobic material: aqueous liquid interfaces, and the multitude of health, environmental and biotechnological consequences of these activities.

More information about this series at http://www.springer.com/series/13884

Terry J. McGenity
Editor

Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology

With 34 Figures and 9 Tables

Springer

Editor
Terry J. McGenity
School of Biological Sciences
University of Essex
Colchester, UK

ISBN 978-3-030-14784-6 ISBN 978-3-030-14785-3 (eBook)
ISBN 978-3-030-14786-0 (print and electronic bundle)
https://doi.org/10.1007/978-3-030-14785-3

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

I would like to dedicate this book to my mother, Eileen McGenity, who passed away during its conception; she was the epitome of fortitude and love.

Introduction

This book provides comprehensive, authoritative discussions about microbial communities in environments that are rich in hydrocarbons. Together with the other books in this series, it builds on the first edition of the *Handbook of Hydrocarbon and Lipid Microbiology* (Timmis et al. 2010). Commonly the first step in microbial community ecology is to identify which microbes are present in a particular environment, for example, by analyzing phylogenetically informative gene sequences obtained from environmental DNA. This procedure allows estimates of relative abundance of particular phylogenetic groups, and thus comparison over time and space, between contaminated and uncontaminated environments, and between experimental treatments. Further comparisons with sequences in databases, especially where abundant and high-quality environmental metadata are available, allow patterns of phylotype distribution to emerge, setting up questions about factors influencing microbial distribution. The chapters in this book draw on this core information, but also a range of contemporary approaches that advance our understanding of community assembly, interactions, and activities. This book is complemented by the 17-volume Hydrocarbon and Lipid Microbiology Protocols series, which explains how cultivation-independent methods go beyond surveying communities (McGenity et al. 2017a). A plethora of metagenomic and functional genomic approaches, coupled with stable-isotope methods, can be used to identify active populations and link phylogeny and function in microbial communities (McGenity et al. 2017b), including single-cell (McGenity et al. 2016a) and imaging (McGenity et al. 2016b) methods.

The application of these techniques was particularly prominent in studies that emerged after the Deepwater Horizon oil spill in the Gulf of Mexico. In this book, Redmond and Valentine (2018) paint a succinct and clear picture of how the connections between microbial populations and their activities were established in this unique deep-sea, gas-rich spill, focusing on some of the most abundant taxa: (1) a novel group of alkane-degrading Oceanospirillales; (2) *Cycloclasticus*, known for degrading polycyclic aromatic hydrocarbons, but which also probably oxidizes short-chain alkanes; (3) *Colwellia*, which demonstrated the capacity to degrade both *n*-alkanes and aromatics; and (4) methanotrophs and methylotrophs, the precise function of the latter remaining uncertain.

Staying in the marine environment, but moving to the shore, Goñi-Urriza and Duran (2018) expertly explain how stratified microbial-mat communities couple degradation of petroleum hydrocarbons to different terminal electron acceptors, while being influenced by seasonal and diel cycles. They also highlight how oil can be trapped beneath growing mats, thus restricting its physical and chemical transformation. Within microbial mats and in aquatic environments, the synergistic interactions between microalgal phototrophs and hydrocarbon-degrading bacteria are important in effective biodegradation of hydrocarbons, as articulated in the chapter by Abed (2018).

Cravo-Laureau and Duran (2018) focus on the microbial ecology of marine environments that have been chronically polluted by petroleum, where microbes and their community networks are able to adapt to the presence of hydrocarbons and other pollutants over years or decades. Nogales and Bosch (2018) hone in on harbors and marinas, which are typically chronically polluted with hydrocarbons and a range of other chemicals, including heavy metals. They expose how little we know about the microbial ecology of such man-made environments, and emphasize that hydrocarbon fate is linked to the capacity of microbes to tolerate multiple stressors.

A high proportion, perhaps most (National Research Council 2003), of the oil in the sea comes from natural oil seeps, which is the topic covered in the chapter by Teske (2018), which compares the Gulf of Mexico, where deeply buried petroleum rises through extensive sediment layers, and the hydrothermal Guaymas Basin in the Gulf of California, where buried organic matter is transformed into young petroleum within the upper part of the sediment. Teske (2018) concentrates on anaerobic hydrocarbon degradation processes and the microbial communities responsible. In the following chapter, Knittel et al. (2018) consider the anaerobic oxidation of the smallest hydrocarbon, methane, focusing on the mechanism of the most well-established processes involving anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB).

The chapter by Verbeke et al. (2018) also discusses methane oxidation (both aerobic and anaerobic), with an emphasis on acidic, northern peatlands, an ecosystem that is extremely susceptible to global-warming-induced increases in methane emissions. The chapter emphasizes the taxonomy and physiology of active methanotrophs in these environments, including the established methanotrophs from the Alphaproteobacteria and Gammaproteobacteria, but also the Verrucomicrobia, which are commonly associated with geothermal environments, and upland soil cluster alpha (USCα), a yet-to-be cultivated group that has a high affinity for methane.

Deserts are one of the most extensive terrestrial biomes, and are frequently subjected to pollution by crude oil, especially in the Arabian Peninsula, owing to a combination of conflict and large-scale oil production. Muthukrishnan and Abed (2018) provide a comprehensive review of petroleum hydrocarbon degradation in both hot and cold deserts, including a discussion on bioremediation strategies.

Four chapters review the microbiology of less-conventional, but increasingly exploited, fossil-fuel reserves, such as coal environments (Marks and Callaghan

2018), oil shales (kerogen), heavy-oil reservoirs, and bitumen deposits (Gieg 2018), as well as their waste streams, such as tailing ponds (Siddique et al. 2018) and a major class of pollutants found within them, namely, naphthenic acids (Skeels and Whitby 2018). Marks and Callaghan (2018) explain how buried coal beds exposed to formation waters are the sites of anaerobic syntrophic microbial production of methane (both a mining hazard and a cleaner fuel) from coal-derived organic matter, which includes aromatics, alkanes, phenols, naphthenic acids, and N-, S-, and O-containing heterocyclic compounds. In contrast, air-exposed surface coal or its spoil, especially when rich in sulfur, provides the ideal blend of components for microbial acid production that leaches heavy metals into the wider environment (Marks and Callaghan 2018). The chapter by Gieg (2018) highlights the need to better understand the microbial processes in oil shales, heavy-oil, and bitumen deposits, for example, in order to more rationally produce methane from marginal reserves. Gieg (2018) also raises the question as to why so many DNA sequences from aerobic microbes are found in anoxic deep oil sands. The extraction of bitumen from surface oil sands is now performed on an immense scale, resulting in huge volumes of fluid fine tailings that are stored in oil sands tailings ponds (OSTP), as outlined by Siddique et al. (2018). These OSTP are the source of numerous environmental problems, including production and emission of the greenhouse gas methane, and a plethora of inorganic and organic pollutants, notably naphthenic acids and PAHs. Environmental directives, especially in Canada, have led to research into microbial solutions to these problems, which have in turn required a fundamental understanding of biogeochemical cycles in OSTP, which are expertly reviewed by Siddique et al. (2018). Skeels and Whitby (2018) provide a detailed review of biodegradation of the persistent and toxic naphthenic acids, which include acyclic and cyclic, saturated, and aromatic carboxylic acids. They emphasize the need for more studies on complex natural mixtures, rather than single, commercially available naphthenic acids. Although a wide range of species are involved in naphthenic acid biodegradation, *Pseudomonas* species are almost always associated with the process under aerobic conditions (Skeels and Whitby 2018), and are becoming an important model for understanding naphthenic-acid catabolic pathways.

Souring of oil reservoirs is caused by sulfate-reducing microorganisms (SRM) producing hydrogen sulfide, which is toxic and explosive, and can lead to corrosion and air pollution. Thus, there are major imperatives to reduce hydrogen sulfide formation. The chapter by Carlson and Hubert (2019) expertly explain how the addition of nitrate to oil reservoirs enables nitrate-reducing microbes to outcompete SRM for common carbon and energy sources, as well as encouraging nitrate-reducing sulfide-oxidizing bacteria, thereby diminishing the production and buildup of sulfide, respectively. The authors discuss different mechanisms of SRM inhibition and the layers of complexity associated with this process, as well as the diverse methods for microbial monitoring of oil reservoirs to: (1) determine the potential for, or the extent, of souring; (2) guide treatment options; and (3) identify whether treatment is working. Carlson and Hubert (2019) also discuss the more recent

innovation of adding perchlorate as an electron acceptor to encourage bio-competitive exclusion of SRM, as well as early-stage research into the application of other oxyanions.

The final chapter in the book features fungi, a group that is often neglected when it comes to hydrocarbon degradation. Prenafeta-Boldú et al. (2018) provide a convincing discussion about why we should be considering fungal catabolic processes, ranging from co-metabolism of high molecular weight PAHs by the extracellular enzymes (peroxidases and laccases) from lignin-degrading fungi to assimilatory metabolism of certain alkylbenzenes employing cytoplasmic cytochrome P450 monooxygenases. Such fungi are biotechnologically valuable; indeed several alkylbenzene degraders were isolated from biofilters treating toluene and styrene-polluted air. On the other hand, fungi are renowned for biodeterioration of fuels, and have even been implicated in aircraft accidents. The number one culprit is considered to be the "kerosene fungus," *Amorphotheca resinae* (Prenafeta-Boldú et al. 2018).

I would like to conclude by expressing my gratitude to the authors, who have provided such wide-ranging and fascinating insights into hydrocarbon-degrading microbial assemblages, covering diverse taxa that degrade a plethora of hydrocarbons (or carry out other important biotransformations) in a range of environments.

School of Biological Sciences
University of Essex, Colchester, UK

Terry J. McGenity

References

Abed RMM (2018) Phototroph-heterotroph oil-degrading partnerships. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_15-1

Carlson HK, Hubert CRJ (2019) Mechanisms and monitoring of oil reservoir souring control by nitrate or perchlorate injection. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_17-1

Cravo-Laureau C, Duran R (2018) Microbial ecology of marine environments chronically polluted by petroleum. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_13-1

Gieg LM (2018) Microbial communities in oil shales, biodegraded and heavy oil reservoirs, and bitumen deposits. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology.

Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_4-1

Goñi-Urriza M, Duran R (2018) Impact of petroleum contamination on microbial mats. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_14-1

Knittel K, Wegener G, Boetius A (2018) Anaerobic methane oxidizers. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_7-1

Marks CR, Callaghan AV (2018) Surface and subsurface coal environments: from environmental formation and chemistry to microbial communities. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_9-1

McGenity TJ, Timmis KN, Nogales B (eds) (2016a) Hydrocarbon and lipid microbiology protocols: single-cell and single-molecule methods. Springer, Berlin/Heidelberg

McGenity TJ, Timmis KN, Nogales B (eds) (2016b) Hydrocarbon and lipid microbiology protocols: ultrastructure and imaging. Springer, Berlin/Heidelberg

McGenity TJ, Timmis KN, Nogales B (eds) (2017a) Hydrocarbon and lipid microbiology protocols: microbial quantitation, community profiling and array approaches. Springer, Berlin/Heidelberg

McGenity TJ, Timmis KN, Nogales B (eds) (2017b) Hydrocarbon and lipid microbiology protocols: genetic, genomic and system analyses of communities. Springer, Berlin/Heidelberg

Muthukrishnan T, Abed RMM (2018) Microbial communities in hydrocarbon-contaminated desert soils. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_11-1

National Research Council (2003) Oil in the sea III: inputs, fates and effects. National Academy Press, Washington, DC

Nogales B, Bosch R (2018) Microbial communities in hydrocarbon-polluted harbors and marinas. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_18-1

Prenafeta-Boldú FX, Sybren de Hoog G, Summerbell RC (2018) Fungal communities in hydrocarbon degradation. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_8-2

Redmond MC, Valentine DL (2018) Microbial communities responding to deep-sea hydrocarbon spills. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_12-1

Siddique T, Stasik S, Mohamad Shahimin MF, Wendt-Potthoff K (2018) Microbial communities in oil sands tailings: their implications in biogeochemical processes and tailings management. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_10-1

Skeels K, Whitby C (2018) Microbial ecology of naphthenic acid (NA) degradation. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_5-1

Teske A (2018) Hydrocarbon-degrading microbial communities in natural oil seeps. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_3-1

Timmis KN, McGenity TJ, van der Meer JR, de Lorenzo V (eds) (2010) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin/Heidelberg

Verbeke TJ, Dedysh SN, Dunfield PF (2018) Methanotrophy in acidic soils, including northern peatlands. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: members, metagenomics and ecophysiology. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_6-1

Acknowledgments

It has been a pleasure to have worked with the chapter authors, who have provided fascinating, comprehensive, and authoritative insights into microbial communities inhabiting, and active in, a range of environments with high levels of hydrocarbons and hydrocarbon derivatives. This excellent resource is the result of their expertise, forbearance, and commitment, as well as the support from staff at Springer, the series editor, Ken Timmis, and the reviewers, who have shared their expertise so generously.

Contents

About the Series Editor-in-Chief

Kenneth N. Timmis
Emeritus Professor
Institute of Microbiology
Technical University Braunschweig
Braunschweig, Germany

Kenneth Timmis studied microbiology and obtained his Ph.D. at Bristol University. He undertook postdoctoral training at the Ruhr-University Bochum, Yale and Stanford, at the latter two as a Fellow of the Helen Hay Whitney Foundation. He was then appointed Head of an independent research group at the Max Planck Institute for Molecular Genetics in Berlin and subsequently Professor of Biochemistry in the University of Geneva, Faculty of Medicine. Thereafter, for almost 20 years, he was Director of the Division of Microbiology at the National Research Centre for Biotechnology (GBF)/now the Helmholtz Centre for Infection Research (HZI), and concomitantly Professor of Microbiology in the Institute of Microbiology of the Technical University Braunschweig. He is currently Emeritus Professor in this institute.

The Editor-in-Chief has worked for more than 30 years in the area of environmental microbiology and biotechnology, has published over 400 papers in international journals, and is an ISI Highly Cited Microbiology-100 researcher. His group has worked for many years, inter alia, on the biodegradation of oil hydrocarbons, especially the genetics and regulation of toluene degradation, and on the ecology of hydrocarbon-degrading microbial communities, discovered the new group of marine oil-degrading hydrocarbonoclastic bacteria, initiated genome sequencing

projects on bacteria that are paradigms of microbes that degrade organic compounds (*Pseudomonas putida* and *Alcanivorax borkumensis*), and pioneered the topic of experimental evolution of novel catabolic activities.

He is Fellow of the Royal Society, Member of the European Molecular Biology Organisation, Fellow of the American Academy of Microbiology, Member of the European Academy of Microbiology, and Recipient of the Erwin Schrödinger Prize. He is the founder and Editor-in-Chief of the journals *Environmental Microbiology*, *Environmental Microbiology Reports*, and *Microbial Biotechnology*.

About the Editor

Terry McGenity is a Reader at the University of Essex, UK. His Ph.D., investigating the microbial ecology of ancient salt deposits (University of Leicester), was followed by postdoctoral positions at the Japan Marine Science and Technology Centre (JAMSTEC, Yokosuka) and the Postgraduate Research Institute for Sedimentology (University of Reading). His overarching research interest is to understand how microbial communities function and interact to influence major biogeochemical processes. He worked as a postdoc with Ken Timmis at the University of Essex, where he was inspired to investigate microbial interactions with hydrocarbons at multiple scales, from communities to cells, and as both a source of food and stress. He has broad interests in microbial ecology and diversity, particularly with respect to carbon cycling (especially the second most abundantly produced hydrocarbon in the atmosphere, isoprene), and is driven to better understand how microbes cope with, or flourish, in hypersaline and poly-extreme environments.

Contributors

Raeid M. M. Abed College of Science, Biology Department, Sultan Qaboos University, Muscat, Sultanate of Oman

A. Boetius Max Planck Institute for Marine Microbiology, Bremen, Germany

MARUM, Center for Marine Environmental Sciences, Bremen, Germany

Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany

Rafael Bosch Departamento de Biología, Universidad de las Illes Balears, Palma, Mallorca, Spain

Instituto Mediterráneo de Estudios Avanzados (IMEDEA, UIB-CSIC), Esporles, Mallorca, Spain

Amy V. Callaghan Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA

Hans K. Carlson Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Lab, Berkeley, CA, USA

Cristiana Cravo-Laureau Equipe Environnement et Microbiologie, MELODY group, Université de Pau et des Pays de l'Adour, IPREM UMR CNRS 5254, Pau, France

G. Sybren de Hoog Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

Center of Expertise in Mycology of RadboudUMC / Canisius Wilhelmina Hospital, Radboud University, Nijmegen, The Netherlands

Svetlana N. Dedysh S. N. Winogradsky Institute of Microbiology, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia

Peter F. Dunfield Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

Robert Duran Equipe Environnement et Microbiologie, MELODY group, Université de Pau et des Pays de l'Adour, IPREM UMR CNRS 5254, Pau, France

Lisa M. Gieg Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

Marisol Goñi-Urriza Environmental Microbiology CNRS/ UNIV PAU & PAYS ADOUR, IPREM, UMR5254, Université de Pau et des Pays de l'Adour, Pau, France

Casey R. J. Hubert Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

K. Knittel Max Planck Institute for Marine Microbiology, Bremen, Germany

Christopher R. Marks U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Groundwater, Watershed, and Ecosystem Restoration Division, Ada, OK, USA

Mohd Faidz Mohamad Shahimin Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada

Department of Chemical Engineering Technology, Universiti Malaysia Perlis, Padang Besar, Perlis, Malaysia

Thirumahal Muthukrishnan College of Science, Biology Department, Sultan Qaboos University, Muscat, Sultanate of Oman

Balbina Nogales Departamento de Biología, Universidad de las Illes Balears, Palma, Mallorca, Spain

Instituto Mediterráneo de Estudios Avanzados (IMEDEA, UIB-CSIC), Esporles, Mallorca, Spain

Francesc X. Prenafeta-Boldú Integral Management of Organic Waste (GIRO), Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Catalonia, Spain

Molly C. Redmond Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC, USA

Tariq Siddique Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada

Karl Skeels School of Biological Sciences, University of Essex, Essex, UK

Sebastian Stasik Department of Lake Research, UFZ – Helmholtz Centre for Environmental Research, Magdeburg, Germany

Faculty of Medicine Carl Gustav Carus, Technische Universität, Dresden, Germany

Richard C. Summerbell Sporometrics Inc, Toronto, ON, Canada

University of Toronto, Dalla Lana School of Public Health, Toronto, ON, Canada

Andreas Teske Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

David L. Valentine Department of Earth Science and Marine Science Institute, University of California, Santa Barbara, CA, USA

Tobin J. Verbeke Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

Gunter Wegener Max Planck Institute for Marine Microbiology, Bremen, Germany

MARUM, Center for Marine Environmental Sciences, Bremen, Germany

Katrin Wendt-Potthoff Department of Lake Research, UFZ – Helmholtz Centre for Environmental Research, Magdeburg, Germany

Corinne Whitby School of Biological Sciences, University of Essex, Essex, UK

Microbial Communities Responding to Deep-Sea Hydrocarbon Spills

1

Molly C. Redmond and David L. Valentine

Contents

Abstract

The 2010 Deepwater Horizon oil spill in the Gulf of Mexico can be considered the world's first deep-sea hydrocarbon spill. Deep-sea hydrocarbon spills occur in

M. C. Redmond (✉)
Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC, USA
e-mail: mcredmond@uncc.edu

D. L. Valentine
Department of Earth Science and Marine Science Institute, University of California, Santa Barbara, CA, USA
e-mail: valentine@ucsb.edu

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_12

a different setting than surface oil spills, and the organisms that respond must be adapted to this low-temperature, high-pressure environment. The hydrocarbon composition can also be quite different than at the sea surface, with high concentrations of dissolved hydrocarbons, including natural gas, and suspended droplets of petroleum. We discuss the bacteria that may respond to these spills and factors that affect their abundance, based on data collected during the Deepwater Horizon spill and in microcosm experiments in the following years.

1 Introduction

When the Deepwater Horizon mobile offshore drilling unit exploded on April 20, 2010 and sank 2 days later, it caused the world's first major deep-sea oil spill. Previous well blowouts such as Ixtoc I in the Gulf Mexico in 1979 and Platform A in the Santa Barbara Channel in 1969 also caused significant undersea spills, but the shallower depths of these spills (50–60 m) resulted in most of oil reaching the sea surface. In contrast, the Deepwater Horizon well was 1500 m below the sea surface, and ~25% of the hydrocarbons emitted remained dissolved or suspended in a deep-sea intrusion layer at depths between 900 and 1300 m (Ryerson et al. 2012; Gros et al. 2017). This intrusion layer, commonly referred to as the hydrocarbon plume, created an unusual set of conditions for the microbial communities that responded to this spill. Compared to a sea-surface oil spill, there was a different mix of hydrocarbon compounds, lower temperatures, higher pressure, abundant nutrients, and complete darkness. These differences in environmental conditions led to the development of microbial communities quite different from those observed in previous oil spills, dominated at different points in space and time by a novel *Oceanospirillales* (referred to as DWH *Oceanospirillales*), *Colwellia*, and *Cycloclasticus* (Hazen et al. 2010; Redmond and Valentine 2012). In this chapter, we discuss the environmental features of deep-sea spills that affect microbial communities, changes in microbial community composition during deep-sea spills, and notable members of those communities.

2 Features of Deep-Sea Hydrocarbon Spills

2.1 Composition of Hydrocarbons in the Water Column

The formation of a deep-sea hydrocarbon plume was one of the most distinctive features of the Deepwater Horizon spill and had a significant effect on the microbial communities that developed, so we focus our discussion primarily on hydrocarbon degradation within this plume layer. The deep-sea plume was enriched in the most soluble hydrocarbons including methane, ethane, propane, butanes, and pentanes (C1–C5 alkanes); cyclopentane, methylcyclopentane, cyclohexane, and

methylcyclohexane (C5–C7 cycloalkanes); benzene, toluene, ethylbenzene, and xylenes (BTEX); and naphthalene, methylnaphthalenes, dimethylnaphthalenes, and fluorene (small PAHs), which dissolved according to their aqueous solubilities (Ryerson et al. 2012). In a surface oil spill, many of these volatile compounds would be rapidly lost to the atmosphere rather than available for consumption by microbes, likely a major factor affecting which organisms responded to the spill. In addition to dissolved hydrocarbons, the plume contained suspended petroleum microdroplets, though the amount may have varied over time due to interventions at the wellhead. Between April 22 and June 3, oil was released at two or more points along the riser pipe. After the riser was cut, the Top Hat containment device began to capture oil, and chemical dispersants were applied consistently at the point of oil release, and the composition of the microbial community changed (Dubinsky et al. 2013). These observations suggest that hydrocarbon flux rate and the hydrocarbon composition of the plumes impacted microbial community composition, while also highlighting the potential effect of response efforts on biodegradation and the challenges in predicting microbial community response in future spills.

2.2 Dispersant

One of the most controversial aspects of the Deepwater Horizon spill was the use of chemical dispersants, particularly their unprecedented use in the deep sea. Corexit EC9500A was injected directly into the oil and gas emanating from the fallen riser and later the blowout preventer at the wellhead. The application of dispersant decreased petroleum droplet size by approximately threefold and slowed their rise through the water column, increasing dissolution of water soluble compounds into the deep-sea plume by 25% (Gros et al. 2017) and shifting the area of oil surfacing away from response vessels. This strategy appears to have been effective at reducing the risks of VOC exposure to workers at the sea surface, but dispersant's effects on biodegradation remain a point of contention (Kleindienst et al. 2016b; Prince et al. 2016a, 2017). Here, we focus primarily on its effect on microbial community composition. Based on closed-system microcosm experiments, Kleindienst et al. suggested that dispersant can alter community composition by selecting for organisms like *Colwellia* that are stimulated by dispersant alone and selecting against other hydrocarbon degraders like *Marinobacter* and ultimately may inhibit degradation (Kleindienst et al. 2015). Other microcosm experiments have found no significant effects of dispersant on community composition and degradation rates (Brakstad et al. 2018) or a positive effect on degradation rate, though the stimulation of *Colwellia* by dispersant alone and inhibition of *Marinobacter* in a subset of incubations at 25 °C were also noted (Techtmann et al. 2017). The growth of *Colwellia* in dispersant only treatments suggests that it may be able to metabolize the dispersant, and *Colwellia* strain RC25 was shown to degrade dioctylsulfosuccinate, a component of Corexit (Chakraborty et al. 2012). However, it is important to note that the deep plumes were an open system and the dispersant

components dissolved and diluted into the ocean (Kujawinski et al. 2011). As such, the environmental relevance of microcosm experiments is somewhat limited, and the primary impact of dispersants on the microbial community was likely indirect by modulating the abundance and molecular distribution of hydrocarbon substrates in the water column.

2.3 Temperature

In temperate areas, one of the most important differences between a deep-sea and a surface oil spill is water temperature. During the Deepwater Horizon oil spill, sea surface temperature in the affected area was nearly 30 °C, while temperature in the hydrocarbon plume at 1100 m was only 6 °C. Temperature has two major effects on biodegradation. First, it affects the physical properties of oil and can alter its bioavailability. For example, the respiration rate by *Alcanivorax borkumensis* SK2 growing on individual *n*-alkanes (C14–C20) drops significantly at the temperature that coincides with the liquid-wax phase transition for each *n*-alkane, clearly indicating that bioavailability plays a role independent of the overall microbial temperature optimum (Lyu et al. 2018). The formation of wax-like particles appears to have led to the deposition of long-chain *n*-alkanes in sediments during the Deepwater Horizon spill and slower than expected degradation relative to typically conserved biomarkers (Bagby et al. 2017).

The second effect of temperature is on microbial physiology and community composition. The effect of temperature on community composition in the Gulf of Mexico was nicely demonstrated by Liu et al. (2017a). In experiments with surface and bottom water inocula incubated in filtered water from each environment at 4 °C and 24 °C, with and without oil, they determined that temperature caused the most variation in community composition (57%). Low temperature particularly favored the development of *Cycloclasticus* and *Pseudoalteromonas*, two organisms that were abundant during the Deepwater Horizon spill (Dubinsky et al. 2013). The effects of temperature on community composition have been noted by several others, with *Colwellia*, *Cycloclasticus*, and members of the *Oceanospirillales* frequently mentioned as abundant in low-temperature communities, though they are not found exclusively at low temperature (Coulon et al. 2007; Techtmann et al. 2017; Lofthus et al. 2018).

2.4 Pressure

Hydrostatic pressure in the ocean increases by 1 atm or 0.101 MPa with each 10 m below the sea surface, resulting in a pressure of ~15 MPa at the depth of the Deepwater Horizon oil spill. While temperature is commonly manipulated in lab microcosm experiments, the equipment required to mimic deep-sea pressures is less commonly available, and the effects of pressure are therefore not frequently tested.

Most studies indicate that biodegradation is slower at higher pressure and that cells grow more slowly (Prince et al. 2016b; Scoma et al. 2016; Marietou et al. 2018). The only study of the effect of pressure on hydrocarbon-degrading community composition in the Gulf of Mexico showed that *Oleispira* was dominant at all pressures, while the groups common during the Deepwater Horizon spill weren't observed in any treatment (Marietou et al. 2018). Additional study is required to determine the role pressure may have played in the abundance of these organisms.

2.5 Nutrients and Oxygen

In contrast to many ocean surface environments, nitrogen and phosphorus were relatively abundant in the deep plume environment and, in combination with mixing dynamics, are unlikely to have limited bacterial growth in the Deepwater Horizon spill. However, limitation of trace metals including iron and copper may have had some effect on the activity of hydrocarbon oxidizers or the communities that developed (Bælum et al. 2012; Joung and Shiller 2013; Crespo-Medina et al. 2014). Methane oxidation may also have been affected by the depletion of light rare earth elements required for the XoxF-type methanol dehydrogenase (Shiller et al. 2017), which could alter cross-feeding interactions between methanotrophs and methylotrophs (Krause et al. 2017). In microcosm experiments, nutrient amendments (nitrate, ammonium, phosphate, and trace metals) did not significantly alter the microbial community, though they did slightly increase degradation rates at later time points (Kleindienst et al. 2015). In the circumstance of Deepwater Horizon, dissolved oxygen was sufficient such that respiration in the water column was not limited by its availability. However, other circumstances may differ, such as in hydrocarbons deposited to sediment, and discharge to low-oxygen waters, such as in the Pacific Ocean's oxygen minimum zone.

3 Microbial Community Changes During Deep-Sea Hydrocarbon Spills

Given that the only one major deep-sea oil spill has occurred to date, we focus here on community changes observed during the 2010 Deepwater Horizon spill in the Gulf of Mexico but also discuss results from microcosm experiments in the Gulf of Mexico and elsewhere.

3.1 Community Changes During the Deepwater Horizon Oil Spill

Prior to the Deepwater Horizon spill, there was very little data on microbial community composition of the water column in the deep Gulf of Mexico. However, several samples were fortuitously collected in the northern Gulf of Mexico in March

2010, including one at collected at 800 m just nine nautical miles from the spill site. These samples were later sequenced and showed a community dominated by the SAR11 clade, other *Alphaproteobacteria*, the SAR406 clade, the SAR324 clade, and a diverse group of *Gammaproteobacteria* (King et al. 2013; Yang et al. 2016). This background community persisted throughout the spill at depths above and below the main plume and returned after the spill ended (Redmond and Valentine 2012; Yang et al. 2016).

The microbial community in the first weeks after the spill began went unsampled, as it took more than a month to obtain resources for sample collection. Several different groups of researchers obtained samples from the deep-sea plume in late May, by which point a novel group of *Oceanospirillales* (referred to as DWH *Oceanospirillales*) had become extremely abundant, dominating 16S rRNA clone libraries, pyrosequencing datasets, metagenomes, and transcriptomes (Hazen et al. 2010; Mason et al. 2012; Redmond and Valentine 2012; Yang et al. 2016). Other samples collected at the end of May showed abundant DWH *Oceanospirillales* but also increasing relative abundance and activity of *Colwellia* and *Cycloclasticus* (Redmond and Valentine 2012; Rivers et al. 2013; Yang et al. 2016). After the riser was cut on June 3, *Colwellia* and *Cycloclasticus* replaced the DWH *Oceanospirillales* as dominant members of the plume community (Valentine et al. 2010; Dubinsky et al. 2013).

The flow of hydrocarbons into the Gulf of Mexico ended July 15, 2010, but sampling efforts were very limited between mid-June and mid-July and sparse throughout the rest of the summer. During this time, the DWH *Oceanospirillales* and then *Colwellia* abundance in plume samples returned to levels found outside of plumes, but *Cycloclasticus* persisted at moderately elevated levels, as did some of the less abundant groups of *Gammaproteobacteria* that had also increased earlier in the spill, such as *Pseudoalteromonas* and *Neptunomonas* (Dubinsky et al. 2013). In August and September, there was also an increase in *Flavobacteria* and *Rhodobacterales* (Redmond and Valentine 2012; Dubinsky et al. 2013). Based on this timing, they were likely consuming the remnants of the primary hydrocarbon degraders, as both groups are commonly associated with the degradation of organic matter in phytoplankton blooms (Buchan et al. 2014), but they may have the ability to directly degrade hydrocarbons as well (Guibert et al. 2016; Hu et al. 2017). The abundance of methanotrophs began to increase in mid-June, followed by methylotrophs (Dubinsky et al. 2013). Both groups were still detectable in plume samples in September 2010, despite the fact that methane concentrations had decreased to below background levels for the Gulf of Mexico (Kessler et al. 2011).

3.2 Microcosm Studies

Since the 2010 spill, several studies have used microcosms to study the effects of hydrocarbons on microbial community composition. These studies vary in their ability to replicate in situ conditions but allow the controlled manipulation of

temperature, pressure, nutrients, dispersant, and other factors. They can also be coupled with detailed measurements of hydrocarbon degradation and techniques like stable-isotope probing (SIP) to identify the organisms consuming specific hydrocarbon substrates, as well as metagenomic sequencing. It should be noted that not all of these studies were conducted with water collected from the deep sea but may still provide insight into hydrocarbon-degrading communities in low-temperature environments or organisms that play an important role in the deep sea. These microcosm studies consistently show similar changes in community composition as observed during the Deepwater Horizon oil spill: an increase in *Gammaproteobacteria*, especially *Cycloclasticus*, *Alteromonadales* (including *Colwellia*), *Oceanospirillales,* and often *Flavobacteria* and *Rhodobacterales* (Brakstad et al. 2015; Kleindienst et al. 2015; Hu et al. 2017; Liu et al. 2017a; Techtmann et al. 2017; Ribicic et al. 2018b). However, the specific organisms within the orders *Alteromonadales* and *Oceanospirillales* often vary, and the DWH *Oceanospirillales* only rarely appear.

4 Organisms

There were four major groups of bacteria that responded to the Deepwater Horizon oil spill: the DWH *Oceanospirillales*, *Cycloclasticus*, *Colwellia*, and methanotrophs/methylotrophs. Relationships between these four groups are shown in a phylogenetic tree of 16S rRNA gene sequences in Fig. 1. We discuss the role of each here.

4.1 DWH Oceanospirillales

The order *Oceanospirillales* contains a number of hydrocarbon degraders, including those in the genera *Alcanivorax*, *Oleispira*, *Thalassolituus*, *Oleibacter*, and *Halomonas*, but the 16S rRNA gene from the DWH *Oceanospirillales* observed in May 2010 showed just 95% similarity to the closest cultured isolate, *Spongiispira norvegica* (Hazen et al. 2010). Even within uncultured sequences, there were few close matches in GenBank at the time, though they were later reported as having been abundant offshore North Carolina in 2009 (D'Ambrosio et al. 2014; Yang et al. 2016). Oligotyping analysis showed that the DWH *Oceanospirillales* operational taxonomic units (OTUs) that responded during the spill were distinct from those observed in the Gulf of Mexico prior to the spill, suggesting that the Deepwater Horizon spill created an unusual set of conditions for this typically rare organism to rapidly respond (Kleindienst et al. 2016a). Based on its disappearance after the riser was cut in early June, the DWH *Oceanospirillale*s may have been involved in the degradation of specific hydrocarbons that were disproportionately abundant in the early stages of the spill. Attempts at cultivation have been unsuccessful, and though single-cell genomics, metagenomics, and transcriptomics have provided insight,

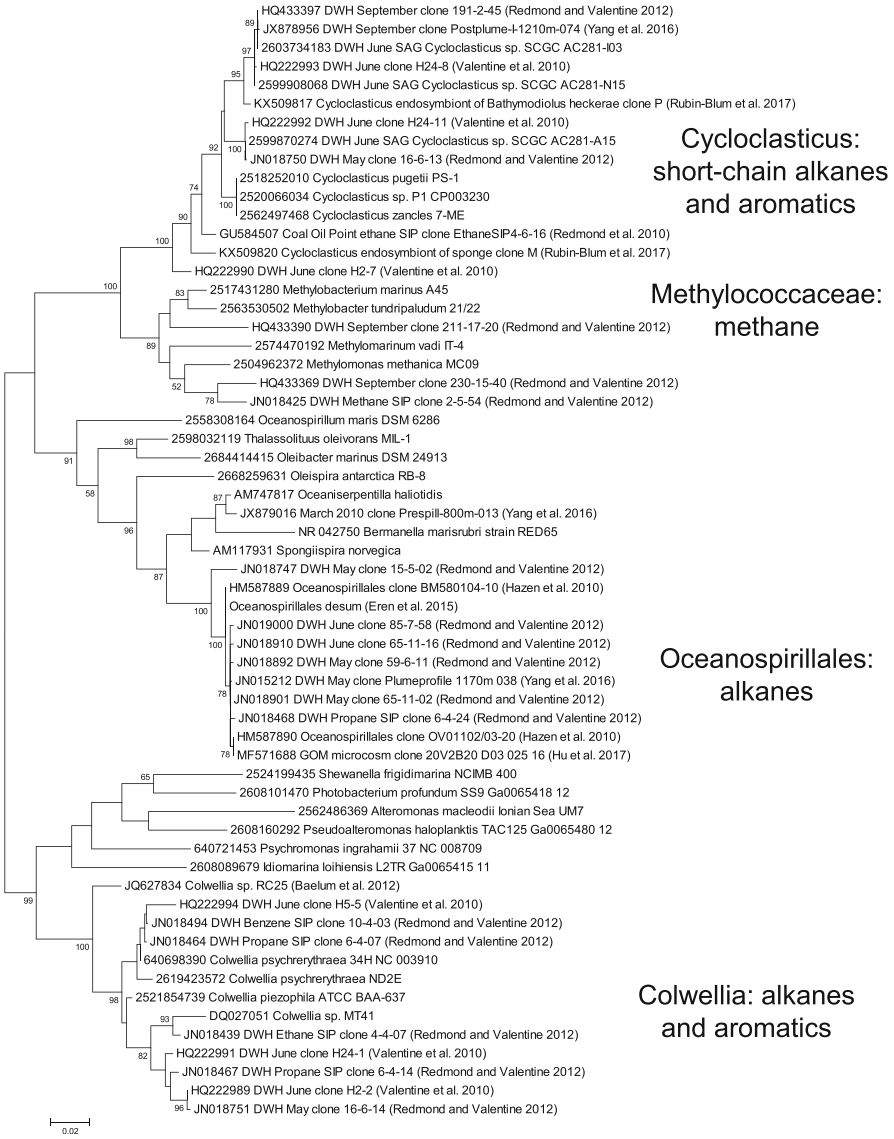

Fig. 1 Maximum likelihood tree of 16S rRNA gene sequences from the major groups of Gammaproteobacteria responding to the Deepwater Horizon oil spill and their cultured relatives. Sequences are identified by GenBank accession number or IMG gene ID

it remains unclear why this previously uncommon hydrocarbon degrader was so abundant in this spill.

During the time that the DWH *Oceanospirillales* were dominant, metagenomes and transcriptomes suggest that alkane oxidation was the primary hydrocarbon degradation process, while genes involved in BTEX degradation were not significant

(Mason et al. 2012). Single-cell-amplified genomes (SAGs) from *Oceanospirillales* cells collected at the same time contained the genes for cyclohexane oxidation (Mason et al. 2012). Cyclohexane was abundant in the deep-sea plume, but not common in sea-surface spills, providing a possible explanation for the high abundance of this organism. However, it should be noted that the 16S rRNA gene sequences from the SAGs were only 95% similar to the dominant DWH *Oceanospirillales* OTU, hinting at some of the challenges of determining which organisms are the "same" when making inferences about function. This is particularly difficult in microcosm experiments, where the differing environmental conditions increase the likelihood of observing organisms that have closely related 16S rRNA gene sequences to those observed in situ but quite different genomes and ability to degrade hydrocarbons.

As mentioned above, the DWH *Oceanospirillales* have generally failed to dominate or sometimes appear at all in microcosm experiments. It remains unclear whether this is due to a specific set of environmental conditions (e.g., high pressure), a preference for particular hydrocarbons that may not be included in the microcosm experiments in sufficient amounts (e.g., cyclohexane), or simply low abundance in many environments. They did increase slightly and appear to take up ^{13}C-labeled ethane and propane in SIP experiments conducted in September 2010 (Redmond et al. 2010) but decreased during microcosm experiments with oil and dispersant from 2013 (Kleindienst et al. 2015). One notable exception was claimed in a recent study by Hu et al. (2017), who attempted to mimic deep-sea plume conditions by dispersing oil into 10 μm diameter droplets. By day 6 of their experiments, 33.5% of metagenome sequences were assigned to a genome bin they termed *Candidatus Bermanella macondoprimitus*, with a 16S rRNA gene sequence nearly identical to those from the Deepwater Horizon oil spill (shown in Fig. 1 as GOM microcosm clone). This genome bin contained just one gene for hydrocarbon degradation, an *alk*B for alkane oxidation (Hu et al. 2017). However, subsequent comparison of this genome bin to metagenomes from the oil spill showed that *Candidatus Bermanella macondoprimitus* was in fact distinct from the organism abundant during the Deepwater Horizon spill and the *alk*B gene was not found in the Deepwater Horizon plume metagenomes (Eren et al. 2015; Delmont and Eren 2017). While the DWH *Oceanospirillales* were almost certainly involved in alkane oxidation, their precise substrate preference, the relative importance of cycloalkanes vs. *n*-alkanes, and variation between individuals remains unclear.

4.2 Cycloclasticus

Unlike the DWH *Oceanospirillales* and *Colwellia*, *Cycloclasticus* was already well known as one of the most abundant marine hydrocarbon-degrading bacteria, believed to mostly consume polycyclic aromatic hydrocarbons (PAHs) (Kasai et al. 2002; Maruyama et al. 2003; McKew et al. 2007; Wang et al. 2008). *Cycloclasticus* was detected in the earliest plume samples and persisted throughout the course of the spill (Dubinsky et al. 2013). It was still readily detectable in plume

remnant samples in September 2010, suggesting that it was indeed consuming the more recalcitrant PAH compounds remaining at that point (Redmond and Valentine 2012). However, its much higher abundance earlier in the summer was likely due to its ability to oxidize ethane and propane. SAGs from June 2010 show that *Cycloclasticus* was abundant when ethane and propane oxidation rates were high (Valentine et al. 2010) and contained short-chain hydrocarbon monooxygenases as well as the rest of the genes required for ethane and propane oxidation (Rubin-Blum et al. 2017). In a separate metatranscriptomic study, these hydrocarbon monooxygenases were some of the most highly expressed genes (Rivers et al. 2013), suggesting ethane and propane oxidation played a role in their rapid growth early in the spill.

It remains unclear whether short-chain alkane oxidation is common in *Cycloclasticus* and how this process affects or is affected by PAH degradation. Cultured *Cycloclasticus* were isolated as PAH degraders and lack the hydrocarbon monooxygenase genes detected in the SAGs (Lai et al. 2012; Cui et al. 2013; Messina et al. 2016). However, similar genes were observed in metagenome assembled genomes (MAGs) from the *Cycloclasticus* symbionts of mussels and sponges in the Gulf of Mexico (Rubin-Blum et al. 2017) and MAGs from *Cycloclasticus* in microcosm studies conducted in the Gulf of Mexico several years after the Deepwater Horizon spill (Hu et al. 2017). *Cycloclasticus* 16S rRNA gene sequences and similar hydrocarbon monooxygenase genes were also detected in ethane SIP experiments at hydrocarbon seep sediments in the Coal Oil Point seep field offshore Santa Barbara, CA (Redmond et al. 2010), so short-chain alkane oxidation may be common in *Cycloclasticus* living in regions where hydrocarbon seeps release both oil and natural gas. Interestingly, the mussel and sponge symbionts appear to have lost the genes for PAH degradation, leaving them dependent on ethane and propane oxidation, whereas the water column SAGs and MAGs from the oil-spill and microcosm experiments retain genes for PAH oxidation (Hu et al. 2017; Rubin-Blum et al. 2017).

4.3 Colwellia

Prior to the Deepwater Horizon spill, *Colwellia* had not been widely recognized as an important hydrocarbon degrader, though it had been identified in oil-contaminated Arctic sea ice and Antarctic seawater sediments (Powell et al. 2004; Yakimov et al. 2004; Brakstad et al. 2008). *Colwellia psychrerythraea* had been studied as a model of psychrophily, with isolates unable to grow above 20 °C (Methé et al. 2005), and the low temperature appears to have played a role in its dominance during the Deepwater Horizon spill (Redmond and Valentine 2012). It was abundant throughout May and June, especially after the decrease in the DWH *Oceanospirillales*. SIP suggested that *Colwellia* was involved in ethane, propane, and benzene oxidation, though it was also capable of growing on crude oil as the sole carbon source (Redmond and Valentine 2012). Other studies have shown that a *Colwellia* isolate

was able to degrade *n*-alkanes and hydrocarbon components of dispersant (Bælum et al. 2012) and that they incorporated ^{13}C-phenanthrene (Gutierrez et al. 2013), indicating that the genus has the potential for widespread hydrocarbon metabolism. It has since been observed in many microcosm studies conducted with low temperature seawater from around the world and linked to the degradation of both *n*-alkanes and aromatics (Brakstad et al. 2015; Kleindienst et al. 2015; Campeão et al. 2017; Hu et al. 2017; Bacosa et al. 2018; Lofthus et al. 2018; Ribicic et al. 2018a).

4.4 Methanotrophs and Methylotrophs

Methane was the most abundant compound released during the Deepwater Horizon spill, accounting for 15% of the total mass of hydrocarbons released (Reddy et al. 2012). It dissolved completely into the deep-sea hydrocarbon plume, where it made up approximately 60% of the mass of soluble hydrocarbons in the plume (Ryerson et al. 2012), but methanotrophs were much slower to respond than the other hydrocarbon degraders (Redmond and Valentine 2012; Dubinsky et al. 2013). In June, methane oxidation rates were much lower than ethane and propane oxidation rates, and known methane oxidizers weren't detected in 16S rRNA gene clone libraries (Valentine et al. 2010). However, between June and September, methane was completely consumed, leaving a decrease in dissolved oxygen in the hydrocarbon plume and a residual community of methanotrophs and methylotrophs (Kessler et al. 2011). The methanotrophs were *Gammaproteobacteria* from the family *Methylococcaceae*, while the methylotrophs were from the *Gammaproteobacterial* genus *Methylophaga* and the *Betaproteobacterial* family *Methylophilaceae*. There was some debate over the role of the methylotrophs, as they are generally assumed to be capable of consuming methanol and other C1 compounds, but not performing the first step of methane oxidation, the oxidation of methane to methanol (Joye et al. 2011). *Methylophaga* has also been shown to consume hexadecane (Mishamandani et al. 2014) and respond rapidly to the addition of high molecular weight dissolved organic matter (McCarren et al. 2010). Its appearance in microcosm experiments with oil and no methane (Hu et al. 2017) suggests that the presence of methane is not a requirement for growth. However, cross-feeding between methanotrophs and methylotrophs, presumably due to excretion of methanol, has been well documented and may be mediated by the availability of limiting nutrients (Redmond et al. 2010; Beck et al. 2013; Krause et al. 2017; Yu and Chistoserdova 2017). This makes methane oxidation an equally plausible explanation for the presence of *Methylophaga* and especially *Methylophilaceae*, and it seems likely that they acted as facultative methylotrophs incorporating carbon from both methane and petroleum.

The lack of data during the critical time period of methane loss leaves a number of unresolved questions about the response of methanotrophs during deep-sea hydrocarbon spills. Though Valentine et al. measured low methane oxidation rates near the wellhead in mid-June (Valentine et al. 2010), Crespo-Medina et al. detected some

sites with extremely high methane oxidation rates a few weeks earlier (Crespo-Medina et al. 2014). While we suspect the elevated rates measured by Crespo-Medina are partially due to an incubation artifact resulting from an unavoidable 1–2 week delay between sample collection and tracer-rate substrate amendment (Crespo-Medina et al. 2015), they may also be explained by differences in sampling locations and spatial heterogeneity in hydrocarbon distributions and ocean circulation (Valentine et al. 2012). The microbial community data clearly show that methanotrophs were slower to increase than other hydrocarbon degraders and were not abundant in any samples collected in May and early June (Redmond and Valentine 2012; Dubinsky et al. 2013). The dominant "methane" monooxygenase genes measured by Crespo-Medina et al. in May and early June are related to the putative ethane and propane monooxygenases from *Cycloclasticus* (Rubin-Blum et al. 2017), though we know little about their substrate specificity and cannot exclude the possibility that they are also capable of some methane oxidation. Methanotrophs finally began to increase in mid-June (Dubinsky et al. 2013). It's unclear whether the presence of other hydrocarbons or hydrocarbon oxidizers inhibited the growth of methanotrophs or if they were simply slower to respond to the increase in available substrate; methane oxidation has also been shown to lag ethane and propane oxidation at natural hydrocarbon seeps, suggesting this pattern may be common (Mendes et al. 2015). Despite the extended lag time, methane was completely consumed by the end of August (Kessler et al. 2011).

5 Research Needs

Another deep-sea oil spill is inevitable. In order to better predict the response of microbial communities to a future spill, several lines of research would be useful:

- **Is the Gulf of Mexico representative of the deep-sea elsewhere?** The vast majority of the research in response to the Deepwater Horizon spill has been focused on the Gulf of Mexico. Though the Gulf of Mexico is certainly a likely location for a future spill, significant deepwater and ultra-deepwater drilling also occur offshore Norway, Angola, and Brazil, and a tanker spill or large ship wreck could cause an oil spill anywhere in the world. Would a deep-sea oil spill somewhere else result in similar microbial communities and degradation rates? Studies from the Eastern Mediterranean (Liu et al. 2017b) and Amazon basin (Campeão et al. 2017) show some similarities, but more extensive study is needed, particularly in areas without natural seepage to provide a background population of hydrocarbon degraders or where low oxygen or nutrient concentrations limit microbial activity.
- **How does the presence of natural gas affect microbial communities and degradation of petroleum hydrocarbons?** The major PAH degrader in the Deepwater Horizon spill, *Cycloclasticus*, had the genetic potential to metabolize both the short-chain alkanes in natural gas and PAHs in oil (Rubin-Blum et al. 2017).

Ethane and propane oxidation rates were high early in the summer (Valentine et al. 2010) and ethane and propane monooxygenases highly expressed (Rivers et al. 2013), suggesting that *Cycloclasticus* may have preferentially oxidized ethane and propane relative to PAHs. Did this delay the onset of PAH degradation, or did a different group of *Cycloclasticus* initiate growth on PAHs? Alternatively, did ethane and propane stimulate the growth of *Cycloclasticus* and ultimately increase PAH degradation rates?

- **What are the functions of individual members of the hydrocarbon-degrading community?** Many inferences about the function of organisms responding to the Deepwater Horizon spill were made through culture-independent techniques such as single-cell and metagenomic sequencing, which will become even more common in future oil spills. However, the ability to make inferences about function from genome sequences is still limited in terms of specificity (e.g., substrate range of an alkane monooxygenase) and likely misses novel or poorly characterized genes involved in hydrocarbon oxidation. Culture-dependent or culture-independent efforts to better link gene sequences to enzyme function would improve such predictions.
- **How does water-column depth impact the microbial response?** Would there be a difference between a spill at 300 m and 3000 m? The difference in temperature and pressure would affect microbial communities directly and would also affect hydrocarbon solubility which could indirectly affect community composition. Nutrient and oxygen availability may vary as well. Microcosm experiments conducted at atmospheric pressure have limited utility in answering this question, and additional efforts should be made to understand the effect of pressure on hydrocarbon-degrading communities.

Acknowledgments DLV was supported by NSF OCE-1756947.

References

Bacosa HP, Erdner DL, Rosenheim BE, Shetty P, Seitz KW, Baker BJ, Liu Z (2018) Hydrocarbon degradation and response of seafloor sediment bacterial community in the northern Gulf of Mexico to light Louisiana sweet crude oil. ISME J. https://doi.org/10.1038/s41396-018-0190-1

Bælum J, Borglin S, Chakraborty R, Fortney JL, Lamendella R, Mason OU, Auer M, Zemla M, Bill M, Conrad ME, Malfatti SA, Tringe SG, Holman H-Y, Hazen TC, Jansson JK (2012) Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. Environ Microbiol 14:2405–2416

Bagby SC, Reddy CM, Aeppli C, Fisher GB, Valentine DL (2017) Persistence and biodegradation of oil at the ocean floor following Deepwater Horizon. Proc Natl Acad Sci U S A 114:E9–E18

Beck DA, Kalyuzhnaya MG, Malfatti S, Tringe SG, del Rio TG, Ivanova N, Lidstrom ME, Chistoserdova L (2013) A metagenomic insight into freshwater methane-utilizing communities and evidence for cooperation between the Methylococcaceae and the Methylophilaceae. PeerJ 1:e23

Brakstad OG, Nonstad I, Faksness L-G, Brandvik PJ (2008) Responses of microbial communities in Arctic sea ice after contamination by crude petroleum oil. Microb Ecol 55:540–552

Brakstad OG, Throne-Holst M, Netzer R, Stoeckel DM, Atlas RM (2015) Microbial communities related to biodegradation of dispersed Macondo oil at low seawater temperature with Norwegian coastal seawater. Microb Biotechnol 8:989–998

Brakstad OG, Ribicic D, Winkler A, Netzer R (2018) Biodegradation of dispersed oil in seawater is not inhibited by a commercial oil spill dispersant. Mar Pollut Bull 129:555–561

Buchan A, LeCleir GR, Gulvik CA, González JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nat Rev Microbiol 12:686–698

Campeão ME, Reis L, Leomil L, de Oliveira L, Otsuki K, Gardinali P, Pelz O, Valle R, Thompson FL, Thompson CC (2017) The deep-sea microbial community from the Amazonian Basin associated with oil degradation. Front Microbiol 8:1019

Chakraborty R, Borglin SE, Dubinsky EA, Andersen GL, Hazen TC (2012) Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico. Front Microbiol 3:357

Coulon F, McKew BA, Osborn AM, McGenity TJ, Timmis KN (2007) Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters. Environ Microbiol 9:177–189

Crespo-Medina M, Meile C, Hunter K, Diercks A, Asper V, Orphan V, Tavormina P, Nigro L, Battles J, Chanton J (2014) The rise and fall of methanotrophy following a deepwater oil-well blowout. Nat Geosci 7:423–427

Crespo-Medina M, Meile CD, Hunter KS, Diercks AR, Asper VL, Orphan VJ, Tavormina PL, Nigro LM, Battles JJ, Chanton JP, Shiller AM, Joung DJ, Amon RMW, Bracco A, Montoya JP, Villareal TA, Wood AM, Joye SB (2015) Addendum: the rise and fall of methanotrophy following a deepwater oil-well blowout. Nat Geosci 8:490

Cui Z, Xu G, Li Q, Gao W, Zheng L (2013) Genome sequence of the pyrene- and fluoranthene-degrading bacterium Cycloclasticus sp. strain PY97M. Genome Announc 1:e00536–e00513

D'Ambrosio L, Ziervogel K, MacGregor B, Teske A, Arnosti C (2014) Composition and enzymatic function of particle-associated and free-living bacteria: a coastal/offshore comparison. ISME J 8:2167–2179

Delmont TO, Eren AM (2017) Simulations predict microbial responses in the environment? This environment disagrees retrospectively. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1712186114

Dubinsky EA, Conrad ME, Chakraborty R, Bill M, Borglin SE, Hollibaugh JT, Mason OU, Piceno YM, Reid FC, Stringfellow WT (2013) Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. Environ Sci Technol 47:10860–10867

Eren AM, Esen ÖC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO (2015) Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ 3:e1319

Gros J, Socolofsky SA, Dissanayake AL, Jun I, Zhao L, Boufadel MC, Reddy CM, Arey JS (2017) Petroleum dynamics in the sea and influence of subsea dispersant injection during Deepwater Horizon. Proc Natl Acad Sci U S A 114:10065–10070

Guibert LM, Loviso CL, Borglin S, Jansson JK, Dionisi HM, Lozada M (2016) Diverse bacterial groups contribute to the alkane degradation potential of chronically polluted subantarctic coastal sediments. Microb Ecol 71:100–112

Gutierrez T, Singleton DR, Berry D, Yang T, Aitken MD, Teske A (2013) Hydrocarbon-degrading bacteria enriched by the Deepwater Horizon oil spill identified by cultivation and DNA-SIP. ISME J 7:2091–2104

Hazen T, Dubinsky E, DeSantis T, Andersen G, Piceno Y, Singh N, Jansson J, Probst A, Borglin S, Fortney J (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330:204–208

Hu P, Dubinsky EA, Probst AJ, Wang J, Sieber CMK, Tom LM, Gardinali PR, Banfield JF, Atlas RM, Andersen GL (2017) Simulation of Deepwater Horizon oil plume reveals substrate specialization within a complex community of hydrocarbon degraders. Proc Natl Acad Sci U S A 114:7432–7437

Joung D, Shiller AM (2013) Trace element distributions in the water column near the Deepwater Horizon well blowout. Environ Sci Technol 47:2161–2168

Joye SB, Leifer I, MacDonald IR, Chanton JP, Meile CD, Teske AP, Kostka JE, Chistoserdova L, Coffin R, Hollander D, Kastner M, Montoya JP, Rehder G, Solomon E, Treude T, Villareal TA (2011) Comment on "A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico". Science 332:1033–1033

Kasai Y, Kishira H, Harayama S (2002) Bacteria belonging to the genus Cycloclasticus play a primary role in the degradation of aromatic hydrocarbons released in a marine environment. Appl Environ Microbiol 68:5625–5633

Kessler JD, Valentine DL, Redmond MC, Du M, Chan EW, Mendes SD, Quiroz EW, Villanueva CJ, Shusta SS, Werra LM (2011) A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. Science 331:312–315

King G, Smith C, Tolar B, Hollibaugh J (2013) Analysis of composition and structure of coastal to mesopelagic bacterioplankton communities in the Northern Gulf of Mexico. Front Microbiol 3:438

Kleindienst S, Seidel M, Ziervogel K, Grim S, Loftis K, Harrison S, Malkin SY, Perkins MJ, Field J, Sogin ML, Dittmar T, Passow U, Medeiros PM, Joye SB (2015) Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. Proc Natl Acad Sci U S A 112:14900–14905

Kleindienst S, Grim S, Sogin M, Bracco A, Crespo-Medina M, Joye SB (2016a) Diverse, rare microbial taxa responded to the Deepwater Horizon deep-sea hydrocarbon plume. ISME J 10:400–415

Kleindienst S, Seidel M, Ziervogel K, Grim S, Loftis K, Harrison S, Malkin SY, Perkins MJ, Field J, Sogin ML, Dittmar T, Passow U, Medeiros P, Joye SB (2016b) Reply to Prince et al.: ability of chemical dispersants to reduce oil spill impacts remains unclear. Proc Natl Acad Sci U S A 113:E1422–E1423

Krause SMB, Johnson T, Samadhi Karunaratne Y, Fu Y, Beck DAC, Chistoserdova L, Lidstrom ME (2017) Lanthanide-dependent cross-feeding of methane-derived carbon is linked by microbial community interactions. Proc Natl Acad Sci U S A 114:358–363

Kujawinski EB, Kido Soule MC, Valentine DL, Boysen AK, Longnecker K, Redmond MC (2011) Fate of dispersants associated with the Deepwater Horizon oil spill. Environ Sci Technol 45:1298–1306

Lai Q, Li W, Wang B, Yu Z, Shao Z (2012) Complete genome sequence of the pyrene-degrading bacterium Cycloclasticus sp. strain P1. J Bacteriol 194:6677

Liu J, Bacosa HP, Liu Z (2017a) Potential environmental factors affecting oil-degrading bacterial populations in deep and surface waters of the Northern Gulf of Mexico. Front Microbiol 7:2131

Liu J, Techtmann SM, Woo HL, Ning D, Fortney JL, Hazen TC (2017b) Rapid response of Eastern Mediterranean deep sea microbial communities to oil. Sci Rep 7:5762

Lofthus S, Netzer R, Lewin AS, Heggeset TM, Haugen T, Brakstad OG (2018) Biodegradation of n-alkanes on oil–seawater interfaces at different temperatures and microbial communities associated with the degradation. Biodegradation 29:141–157

Lyu L-N, Ding H, Cui Z, Valentine DL (2018) The wax–liquid transition modulates hydrocarbon respiration rates in Alcanivorax borkumensis SK2. Environ Sci Technol Lett 5:277–282

Marietou A, Chastain R, Beulig F, Scoma A, Hazen TC, Bartlett DH (2018) The effect of hydrostatic pressure on enrichments of hydrocarbon degrading microbes from the Gulf of Mexico following the Deepwater Horizon oil spill. Front Microbiol 9:808

Maruyama A, Ishiwata H, Kitamura K, Sunamura M, Fujita T, Matsuo M, Higashihara T (2003) Dynamics of microbial populations and strong selection for Cycloclasticus pugetii following the Nakhodka oil spill. Microb Ecol 46:442–453

Mason OU, Hazen TC, Borglin S, Chain PS, Dubinsky EA, Fortney JL, Han J, Holman H-YN, Hultman J, Lamendella R (2012) Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. ISME J 6:1715–1727

McCarren J, Becker JW, Repeta DJ, Shi Y, Young CR, Malmstrom RR, Chisholm SW, DeLong EF (2010) Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc Natl Acad Sci U S A 107:16420–16427

McKew B, Coulon F, Osborn A, Timmis K, McGenity T (2007) Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK. Environ Microbiol 9:165–176

Mendes SD, Redmond MC, Voigritter K, Perez C, Scarlett R, Valentine DL (2015) Marine microbes rapidly adapt to consume ethane, propane, and butane within the dissolved hydrocarbon plume of a natural seep. J Geophys Res Oceans 120:1937–1953

Messina E, Denaro R, Crisafi F, Smedile F, Cappello S, Genovese M, Genovese L, Giuliano L, Russo D, Ferrer M, Golyshin P, Yakimov MM (2016) Genome sequence of obligate marine polycyclic aromatic hydrocarbons-degrading bacterium Cycloclasticus sp. 78-ME, isolated from petroleum deposits of the sunken tanker Amoco Milford Haven, Mediterranean Sea. Mar Genomics 25:11–13

Methé B, Nelson K, Deming J, Momen B, Melamud E, Zhang X, Moult J, Madupu R, Nelson W, Dodson R (2005) The psychrophilic lifestyle as revealed by the genome sequence of Colwellia psychrerythraea 34H through genomic and proteomic analyses. Proc Natl Acad Sci U S A 102:10913–10918

Mishamandani S, Gutierrez T, Aitken M (2014) DNA-based stable isotope probing coupled with cultivation methods implicates Methylophaga in hydrocarbon degradation. Front Microbiol 5:76

Powell S, Bowman J, Snape I (2004) Degradation of nonane by bacteria from Antarctic marine sediment. Polar Biol 27:573–578

Prince RC, Coolbaugh TS, Parkerton TF (2016a) Oil dispersants do facilitate biodegradation of spilled oil. Proc Natl Acad Sci U S A 113:E1421–E1421

Prince RC, Nash GW, Hill SJ (2016b) The biodegradation of crude oil in the deep ocean. Mar Pollut Bull 111:354–357

Prince RC, Butler JD, Redman AD (2017) The rate of crude oil biodegradation in the sea. Environ Sci Technol 51:1278–1284

Reddy CM, Arey JS, Seewald JS, Sylva SP, Lemkau KL, Nelson RK, Carmichael CA, McIntyre CP, Fenwick J, Ventura GT, Van Mooy BAS, Camilli R (2012) Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. Proc Natl Acad Sci U S A 109:20229–20234

Redmond MC, Valentine DL (2012) Temperature and natural gas structured the microbial community response to the Deepwater Horizon oil spill. Proc Natl Acad Sci U S A 109:20292–20297

Redmond MC, Valentine DL, Sessions AL (2010) Identification of novel methane-, ethane-, and propane-oxidizing bacteria at marine hydrocarbon seeps by stable isotope probing. Appl Environ Microbiol 76:6412–6422

Ribicic D, Netzer R, Hazen TC, Techtmann SM, Drabløs F, Brakstad OG (2018a) Microbial community and metagenome dynamics during biodegradation of dispersed oil reveals potential key-players in cold Norwegian seawater. Mar Pollut Bull 129:370–378

Ribicic D, Netzer R, Winkler A, Brakstad OG (2018b) Microbial communities in seawater from an Arctic and a temperate Norwegian fjord and their potentials for biodegradation of chemically dispersed oil at low seawater temperatures. Mar Pollut Bull 129:308–317

Rivers AR, Sharma S, Tringe SG, Martin J, Joye SB, Moran MA (2013) Transcriptional response of bathypelagic marine bacterioplankton to the Deepwater Horizon oil spill. ISME J 7:2315–2329

Rubin-Blum M, Antony CP, Borowski C, Sayavedra L, Pape T, Sahling H, Bohrmann G, Kleiner M, Redmond MC, Valentine DL, Dubilier N (2017) Short-chain alkanes fuel mussel and sponge Cycloclasticus symbionts from deep-sea gas and oil seeps. Nat Microbiol 2:17093

Ryerson TB, Camilli R, Kessler JD, Kujawinski EB, Reddy CM, Valentine DL, Atlas E, Blake DR, de Gouw J, Meinardi S (2012) Chemical data quantify Deepwater Horizon hydrocarbon flow rate and environmental distribution. Proc Natl Acad Sci U S A 109:20246–20253

Scoma A, Barbato M, Hernandez-Sanabria E, Mapelli F, Daffonchio D, Borin S, Boon N (2016) Microbial oil-degradation under mild hydrostatic pressure (10 MPa): which pathways are impacted in piezosensitive hydrocarbonoclastic bacteria? Sci Rep 6:23526

Shiller AM, Chan EW, Joung DJ, Redmond MC, Kessler JD (2017) Light rare earth element depletion during Deepwater Horizon blowout methanotrophy. Sci Rep 7:10389

Techtmann SM, Zhuang M, Campo P, Holder E, Elk M, Hazen TC, Conmy R, Santo Domingo JW (2017) Corexit 9500 enhances oil biodegradation and changes active bacterial community structure of oil-enriched microcosms. Appl Environ Microbiol 83:e03462–e03416

Valentine DL, Kessler JD, Redmond MC, Mendes SD, Heintz MB, Farwell C, Hu L, Kinnaman FS, Yvon-Lewis S, Du M (2010) Propane respiration jump-starts microbial response to a deep oil spill. Science 330:208–211

Valentine DL, Mezic I, Maćešić S, Črnjarić-Žic N, Ivic S, Hogand P, Fonoberov VA, Loire S (2012) Dynamic auto-inoculation and the microbial ecology of a deep water hydrocarbon irruption. Proc Natl Acad Sci U S A 109:20286–20291

Wang B, Lai Q, Cui Z, Tan T, Shao Z (2008) A pyrene-degrading consortium from deep-sea sediment of the West Pacific and its key member Cycloclasticus sp. P1. Environ Microbiol 10:1948–1963

Yakimov MM, Gentile G, Bruni V, Cappello S, D'Auria G, Golyshin PN, Giuliano L (2004) Crude oil-induced structural shift of coastal bacterial communities of rod bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. FEMS Microbiol Ecol 49:419–432

Yang T, Nigro LM, Gutierrez T, D'Ambrosio L, Joye SB, Highsmith R, Teske A (2016) Pulsed blooms and persistent oil-degrading bacterial populations in the water column during and after the Deepwater Horizon blowout. Deep-Sea Res II Top Stud Oceanogr 129:282–291

Yu Z, Chistoserdova L (2017) Communal metabolism of methane and the rare earth element switch. J Bacteriol 199:e00328-17

Impact of Petroleum Contamination on Microbial Mats

2

Marisol Goñi-Urriza and Robert Duran

Contents

Abstract

Microbial mats are well-structured bacterial communities where the different functional groups strongly interact. They develop on shorelines contaminated after oil spills and have the capacity to grow on the spilled oil. Their crude oil degradation capacity is now well established in laboratory studies, demonstrating efficient degradation of model hydrocarbon compounds. However, their in situ degradation capacities are more controversial. In many cases, it is likely that the mats are trapping oil in deeper anoxic zones, protecting the oil from abiotic

M. Goñi-Urriza (✉)
Environmental Microbiology CNRS/ UNIV PAU & PAYS ADOUR, IPREM, UMR5254, Université de Pau et des Pays de l'Adour, Pau, France
e-mail: marisol.goni@univ-pau.fr

R. Duran
Equipe Environnement et Microbiologie, MELODY group, Université de Pau et des Pays de l'Adour, IPREM UMR CNRS 5254, Pau, France
e-mail: robert.duran@univ-pau.fr

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_14

transformations. In the last decade, high-throughput sequencing approaches have improved our knowledge of the biodiversity of microbial mats allowing us to describe the changes of microbial communities in response to the presence of crude oil. Many strains, belonging to all of the main functional groups found in microbial mats, have been described for their capacity to degrade oil compounds under both oxic and anoxic conditions. Their abundance significantly increases in the mats after an oil input, highlighting the potential of microbial mats for oil degradation. But microbial mats appear, in many cases, ineffective for in situ bioremediation. Nevertheless, ex situ use of mats communities for oil degradation is promising for bioremediation.

1 Introduction: General Description of Microbial Mats

Microbial mats consist of laminated structures (Fig. 1) where microorganisms are organized according to micro-gradients of oxygen, sulfur species, redox potential, and light (Duran and Goñi Urriza 2010). The exceptionally high microbial diversity within a few microns covers a large range of microbial metabolic groups (oxygenic

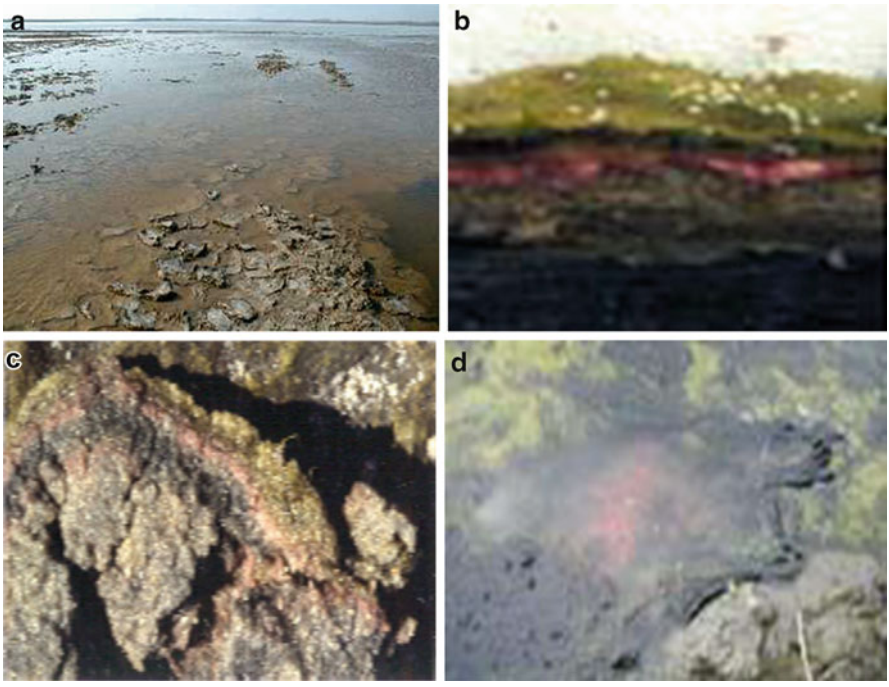

Fig. 1 Microbial mat pictures: (**a**) Field photo microbial mat at Camargue, France. (**b**) Cross section of laminated microbial mat from Camargue, France. Oxygen bubbles produced during photosynthesis are trapped into the microbial mat matrix. (**c**) Cross section of laminated microbial mat from Orkney Island, UK. The active layer corresponds to the top 4 mm. (**d**) Close-up showing flat laminated mat on surface (Guérande, France)

and anoxygenic phototrophs, sulfate reducers, methanogens, etc.). They develop at solid-aqueous interfaces, in environments as diverse as intertidal coastal sediments, hypersaline ponds, alkaline lakes, and thermal springs. Microbial mats have existed since 3.5 billion years ago, which is testament to their resilience and adaptive capacity, largely a consequence of the diversity of interactions between the different functional groups in the community. It is now recognized that microbial mats have influenced Earth's evolution: the oxygen production by photosynthetic microorganisms is believed to be at the origin of the great oxygenation event and eukaryote diversification and colonization of land (Hamilton et al. 2016).

1.1 Organization and Functioning of Microbial Mats

Microbial mats are driven by the activity of photosynthetic microorganisms, which are pioneering the colonization of sediments (Stal et al. 1985). The metabolic capacities of cyanobacteria for carbon dioxide and dinitrogen fixation enrich sediments with organic matter and nutrients, particularly by producing extracellular polymeric substances (EPS). The EPS form a matrix stabilizing sediments (Grant and Gust 1987) and providing a habitat in which microorganisms are embedded and organized according to steep gradients of light, oxygen, and sulfide (Fig. 2). The mature states of these stratified phototrophic communities consist of a thin top layer of sand and/or diatoms covering a dense layer of filamentous cyanobacteria. Below the cyanobacteria, a distinct layer of purple sulfur bacteria is often present with a reduced black layer of precipitated iron sulfides underneath due to intensive sulfate reduction (Van Gemerden 1993). Recent studies, based on high-throughput 16S rRNA gene sequencing, have revealed that microbial mats are among the most diverse marine ecosystems (for a review, see Bolhuis et al. 2014). The hypersaline microbial mats are dominated by cyanobacteria, generally *Microcoleus chthonoplastes* and *Oscillatoria* sp. (Harris et al. 2013). Surprisingly, although cyanobacteria were found in high relative abundance, members of the

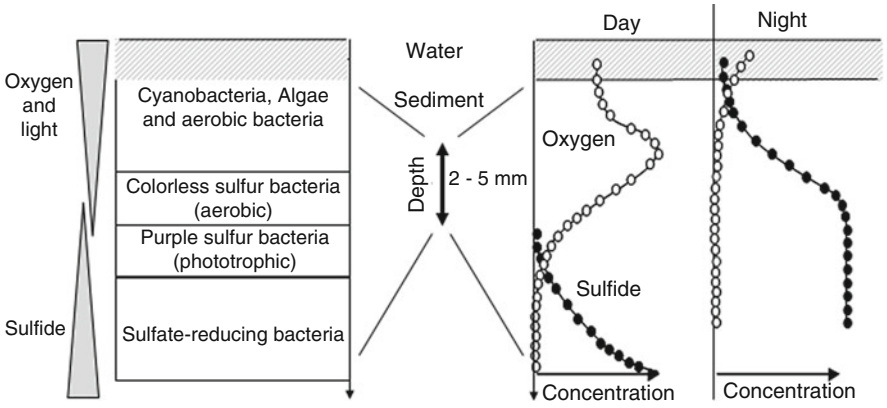

Fig. 2 Microbial mat structure: vertical distribution of functional bacterial groups and steep gradients of light, oxygen, and sulfide

Proteobacteria and *Bacteroidetes* dominated coastal microbial mats (Bolhuis and Stal 2011). However, metatranscriptomic analyses unveiled the dominance of cyanobacteria in the active community, which represented 80–90% of the active populations during a diel cycle, while they represented around 20–40% at the genomic level (Burow et al. 2013; Cardoso et al. 2017). High-throughput sequencing also confirmed the taxonomic separation according to physical-chemical gradients (Kunin et al. 2008). The depth distribution of different populations of bacteria is related to the microenvironmental conditions. Oxygen, light, sulfide, and other (inorganic and organic) sulfur compounds play an important role in the stratification and diversity of these major bacterial groups. Armitage et al. (2012) reported that cyanobacteria were present at 0–2 mm depths, but they dominated the top layer of the mat. In fact, the vertical distributions of the cyanobacteria vary from a homogenous distribution throughout the mat during the night, to a specific localization in the upper oxic and illuminated zone of about 1.5 mm during the day. Solar UV and visible irradiance influence the vertical movements of photosynthetic microorganism such as cyanobacteria and diatoms (Kruschel and Castenholz 1998). Vertical migration has been reported for anoxygenic phototrophic bacteria (Fourçans et al. 2006), sulfate-reducing bacteria (Fourçans et al. 2008), and denitrifying bacteria (Desnues et al. 2007; Fan et al. 2015) in response to the adverse effects of sulfide and oxygen fluxes during a diel cycle. The structure of microbial mats also has seasonal variations, with sulfur-cycle-related bacteria dominating immature mats in spring, while mature mats are dominated by cyanobacteria in the autumn (Aubé et al. 2016; Bolhuis and Stal 2011).

1.2 Analyses of Microbial Mats

Sampling protocols have been developed for the proper collection and preservation of microbial mat sediments in order to conduct a suite of analyses, including chemistry, microscopy, and molecular characterization of microbial communities (for review, see Duran and Cravo-Laureau 2017). The development of suitable techniques has permitted the detailed analysis of these small-scale ecosystems. Particularly, the development of microsensors for oxygen, sulfide, redox potential, pH, and light has allowed the measurements at a fine scale (Abed and Beer 2010; Wieland et al. 2003). Confocal laser scanning microscopy (CLSM) using the natural fluorescence emission of cyanobacteria has been used successfully to identify and quantify each of the different genera separately and to calculate each group's biomass and its space-time dynamic (Diestra et al. 2004). These techniques are now used in combination with fiber-optic microprobe-based spectrometry for determination of phototrophic populations and molecular approaches for characterization of whole or specific bacterial communities. High-throughput sequencing of 16S rRNA genes and metagenomic and metatranscriptomic analyses have been applied to characterize microbial communities and their functioning. The sampling strategies to cover spatial and temporal heterogeneity, DNA extraction methods, and molecular analysis approaches are reviewed by Bolhuis et al. (2014).

1.3 Applications of Microbial Mats

The application of microbial mats has been proposed in various domains including aquaculture, bioremediation of organic compounds, wastewater treatment, metal removal, and production of biohydrogen (for a review, see Roeselers et al. 2008). Microbial-mat-inhabiting microorganisms also present potential applications in biotechnology, for example, by pigment and surfactant production (Guézennec et al. 2011). Most research has addressed the bioremediation potential of microbial mats, particularly regarding their capacities to remove metals, radionuclides, and organic contaminants. Microbial mats sequester and/or precipitate metals/radionuclides by surface sorption or by conditioning the surrounding chemical environment (e.g., elevated pH), thus bio-concentrating the metal/radionuclide. Microbial mats have been shown to degrade and even mineralize a wide spectrum of organic compounds, particularly petroleum hydrocarbon compounds (Abed et al. 2014) and organochloride pesticides (Grötzschel et al. 2004). Microbial mats have been proposed recently for the treatment of saline wastewaters from hydraulic fracturing (Akyon et al. 2015).

2 Microbial Mats and Petroleum Contamination

Active microbial mats usually can develop over highly petroleum-contaminated environments, either in natural seepage zones such as hydrothermal vent of the Guaymas Basin in the Gulf of California (Teske et al. 2014) or in anthropogenically contaminated environments including acute (oil spill accidents) and chronic (petrochemical wastewaters) pollution.

The photosynthetic mats developing on contaminated areas are dominated by populations related to the *Proteobacteria* (mainly *Gamma-* and *Deltaproteobacteria*) and cyanobacteria related to *Lyngbya*, *Phormidium*, *Rivularia*, and *Planktothrix* (Diestra et al. 2004; Mills et al. 2004). *Alpha-*, *Epsilon-*, and *Betaproteobacteria*, *Bacteroidetes* group, *Spirochaetes* phylum, *Verrucomicrobium* group, and *Actinobacteria* phylum are also dominant in polluted mats (Abed et al. 2014; Païssé et al. 2008). Although the groups listed above usually dominate photosynthetic mats, whether they are associated with oil contamination or not (Bolhuis et al. 2014), the oil contamination determines the structure of the bacterial community. Studies that compared bacterial community structure of microbial mats harboring different levels of petroleum contamination demonstrated a correlation of the bacterial diversity with the contamination level (Abed et al. 2014; Païssé et al. 2010). The most polluted mats present particular bacterial community compositions, revealing the adaptation of these communities to the contamination (Hernandez-Raquet et al. 2006; Païssé et al. 2008). In these cases, contamination led to no change in species richness or the dominance of known oil-degrading bacteria (Aubé et al. 2016; Païssé et al. 2008).

Microbial mats developing over petroleum contamination are also affected by seasonal variations. In oil-contaminated mats, cyanobacteria were dominant in all

seasons, indicating a perturbation of the normal seasonal behavior. Such observations suggest that the contamination exerts a selective pressure in the mat's functioning, increasing photosynthesis and respiration (Benthien et al. 2004).

The response of microbial mats to petroleum contamination has been studied in microcosm experiments and in natural conditions, since the second Gulf War led to the largest oil spill in human history in a zone in which many microbial mats developed (Berthe-Corti and Nachtkamp 2010). In 1991, over 770 km of coastline from southern Kuwait to Abu Ali Island (Saudi Arabia) was smothered with oil and tar (Barth 2003), erasing most of the local plant and animal communities (Barth 2002). In the following year, cyanobacteria colonized most of the oil-polluted shores. Three different types of microbial mat development were observed. In one case, desiccation, cracking, and peeling of the cyanobacterial mats were observed, thereby removing the uppermost part of the oiled sediment. The second was the resettlement of burrowing benthic macrofauna such as gastropods and crabs, which disturbed (grazing and burrowing) the cyanobacteria, preventing the mat's development. The third process was further extensive growth of cyanobacteria, building thick laminated mats. These layers completely sealed the surface and overlaid the oil, producing an anaerobic environment in which oil was trapped (Barth 2003). The oil trapped in the anaerobic layers of microbial mats escaped from UV photolysis and the efficient aerobic bacterial degradation, which occurs in the upper layers (Garcia de Oteyza and Grimalt 2006). The impact of petroleum contamination on bacterial communities depends on the pollution history of the mats as observed for other coastal marine environments (Duran and Cravo-Laureau 2016; Duran et al. 2015). The bacterial community structure of pristine mats, according to 16S rRNA gene-based analysis, can persist up to 2 months after an oil spill simulation. However, the structure of the active bacterial community was immediately modified, indicating a rapid microbial mat response. This response was characterized by the dominance of genera from the *Gammaproteobacteria*. *Bacilli* and *Alphaproteobacteria* subsequently appeared in response to petroleum contamination. Be that as it may, the mat recovered its original microbial community structure and metabolically active community 1 year after the contamination (Bordenave et al. 2007). In contrast, in chronically contaminated mats, the bacterial community structure changed after stopping oil input, whereas it remained stable as long as oil was present. The selection pressure created by oil partially determined the bacterial community structure of the mat. Although the supplementary input of petroleum in a chronically contaminated mat did not impact the structure of the community, it immediately impacted the active part of the community, revealed by an apparent decrease of species richness and an increase of the proportion of sequences related to hydrocarbonoclastic strains or petroleum-associated clones in the first hours after the input (Païssé et al. 2010). An induction of dioxygenase (RHD) genes involved in polyaromatic hydrocarbon degradation in the few hours following the oil input was also observed (Païssé et al. 2012). Nevertheless, the decrease of species richness and the induction of RHD genes were quickly reversed, highlighting a fast and efficient adaptive response of the bacterial community. These results suggest that microbial-mat-inhabiting oiled sediments for years adopted a distinctive microbial community

structure that was primed to degrade hydrocarbons rapidly and effectively (Païssé et al. 2010). Although such adaptation allowed a fast response, the dominance of hydrocarbonoclastic bacteria was not observed, and therefore the use of those bacteria as petroleum contamination sentinels should be considered with care, particularly in long-term contaminated environments (Aubé et al. 2016). Whatever the contamination history of the mats, a single oil contamination event leads to the resilience of the community's structure and functioning of the microbial mats in a relatively short period of time.

Petroleum contamination perturbs not only the structure of microbial mats but also their functioning. In laboratory studies, it has been demonstrated that oil contamination stimulates the O_2-consuming processes in microbial mats, probably by the increasing heterotrophic aerobic respiration and sulfate reduction. Higher net and gross photosynthesis rates were observed in long-term oil-polluted mats, which could be a result of an increased availability of CO_2 due to the stimulation of aerobic heterotrophic respiration (Benthien et al. 2004). It has been demonstrated that oil addition stimulates cyanobacterial growth (Chronopoulou et al. 2013) modifying the behavior of cyanobacteria as described previously (Aubé et al. 2016). Oxygen penetration in pristine microbial mats (under light conditions) was up to 3 mm deep, but only 1 mm depth when oil was added (Benthien et al. 2004). For comparison, in darkness, penetration of oxygen was less than 0.5 mm depth (Benthien et al. 2004).

Finally, the response of microbial activities to an oil input was different depending on the season. Pringault et al. (2015) determined the response of microbial mat activities to an input of hydrocarbon-contaminated waters. They compared two microbial mats with different petroleum contamination history in spring and in autumn. In autumn, when a thicker microbial mat was present, no significant changes in oxygen production and respiration were observed after the addition of hydrocarbon-contaminated waters. In contrast, in spring, when a thinner, fluffy mat developed, strong inhibition of both oxygen production and respiration was observed relative to a control without contaminated water. In spring, the decrease in microbial activities was followed by a decrease of the coupling between autotrophs and heterotrophs. The differences observed between the spring and autumn mats might be explained by the maturity of the microbial mat (Pringault et al. 2015).

In summary, active microbial mats are able to develop over the petroleum; they conserve the structure and functioning as found in uncontaminated photosynthetic mats. Pristine mats submitted to a single contamination event respond by resilience in a relative short time. However, in long-term contaminated mats, the bacterial community's structure was dependent on the level of hydrocarbon contamination. Therefore, oil pollution constitutes a selection pressure for these bacterial communities in chronic contaminated mats, which seem well adapted because they maintain their characteristic functioning. The adaptation leads to a fast and effective response to the contamination, followed by a rapid return to a stable equilibrium. This response depends, however, on many different factors, including the stage of maturity of the mats.

3 Hydrocarbon Degradation by Microbial Mats

Although microbial mats have been observed to develop remarkably quickly after oil spills (e.g., after the Gulf War in 1991), their possible role in hydrocarbon degradation is subject to controversy. Laboratory experiments have demonstrated the degradation of crude oil (Abed et al. 2006; Garcia de Oteyza et al. 2006) and model hydrocarbons such as phenanthrene, pristane, octadecane, and dibenzothiophene (Bordenave et al. 2004a, b). Grötzschel et al. (2002) showed that the polyaromatic hydrocarbons (PAH) were preferentially degraded compared to the alkanes. In contrast, Benthien et al. (2004) demonstrated efficient degradation of *n*-alkanes when microbial mats were incubated with crude oil. This degradation occurred together with an increase of aerobic heterotrophic activity. The pristine mats represent an intact and robust ecosystem with the relevant enzymatic capacity such as ring hydroxylating dioxygenases (RHD) for the degradation of PAH (Bordenave et al. 2008; Païssé et al. 2012) and alkane monooxygenases (ALKB) for the degradation of alkanes (Païssé et al. 2011). The gene diversity of these enzymes has been demonstrated to be much higher in polluted mats (Bordenave et al. 2008). Thus, hydrocarbons are mainly degraded under aerobic conditions, with significant involvement of bacterial communities from the upper part of the mats (Abed and Köster 2005; Abed et al. 2002; Chronopoulou et al. 2013; Coulon et al. 2012). The upper part of microbial mats, which is dominated by cyanobacteria and aerobic heterotrophic bacteria, is considered to be the most biologically active layer with respect to carbon cycling. The interactions between cyanobacteria and aerobic heterotrophic bacteria have been demonstrated to play a crucial role in hydrocarbon degradation (Abed 2010). Most hydrocarbonoclastic bacteria isolated from microbial mats have been characterized as diazotrophic bacteria (Al-Mailem et al. 2010).

As microbial mats develop above the oil, they seal the surface and bury oil in anaerobic zones. Hydrocarbon degradation in microbial mats under anoxic conditions has been described involving sulfate-reducing bacteria (Abed et al. 2011). Microbial mats from Saudi Arabia contaminated by crude oil at the end of the 1991 Gulf War were rich in novel halotolerant and thermotolerant microorganisms with the potential to degrade petroleum compounds at elevated salinities and temperatures (Abed et al. 2006, 2007). Nevertheless, chemical analysis showed that the oil-polluted microbial mat exhibited an intermediate degree of transformation between that observed in superficial and deep sediments. Evaporation, photooxidation, and water washing were found to be more effective for hydrocarbon removal than cyanobacteria and its associated microorganisms (Garcia de Oteyza and Grimalt 2006). Furthermore, comparison of some compounds (e.g., regular isoprenoid hydrocarbons or alkylnaphthalenes) in the oil collected in the area after the spill or in the mixtures retained by cyanobacterial growth gave rise to an apparent effect of hydrocarbon preservation in the microbial mat ecosystems. The microbial mats, in these conditions, seemed to prevent oil degradation (Garcia de Oteyza and Grimalt 2006).

3.1 Role of Cyanobacteria in Hydrocarbon Degradation

Crude oil contains inhibitory compounds for cyanobacteria, which reduce enzyme activities and inhibit photosynthesis and growth (Megharaj et al. 2000). The toxic effect of crude oil may induce changes in cyanobacterial species composition in microbial mats, suggesting that any alteration of the community composition could be useful as a bioindicator of pollution. However, cyanobacteria related to members of the genera *Phormidium* and *Planktotrix* were found to have oil tolerance as they were present in mats with a low/medium degree of oil pollution, while *Oscillatoria*-related cyanobacteria were detected in heavily polluted mats (Van Bleijswijk and Muyzer 2004), and cyanobacterial growth has been shown to be stimulated by oil (Chronopoulou et al. 2013; Coulon et al. 2012). Evidence supporting the direct role of cyanobacteria in hydrocarbon degradation is limited, and it is still a matter of discussion whether cyanobacteria from microbial mats participate actively to hydrocarbon biodegradation (Cohen 2002). Sánchez et al. (2006) showed that cyanobacteria were not able to grow utilizing hydrocarbons as carbon and energy sources in packed-column reactor. Similarly, Chaillan et al. (2006) demonstrated that *Phormidium animale*, a cyanobacterium isolated from a heavily contaminated site, was not able to degrade C13–C35 hydrocarbon compounds either in phototrophic (light) or heterotrophic (dark) conditions.

Several studies showed that cyanobacteria play an indirect role in hydrocarbon degradation (for a review, see Ardelean 2014). Abed and Köster (2005) showed that the *Oscillatoria* strain OSC was able to degrade hydrocarbons when associated with aerobic heterotrophic bacteria. Since similar degradation patterns were observed in the light and dark, they concluded that cyanobacteria had no direct effect on hydrocarbon degradation (Cohen 2002), as also demonstrated by Chaillan et al. (2006). It is likely that the degradation activity was exclusively achieved by the other microorganisms present in the microbial consortium of the mat. The cyanobacteria probably played a significant, indirect role in biodegradation by supporting the growth and activity of the actual degraders, providing molecular oxygen for heterotrophic bacteria, organic substances, and surface attachment (Ardelean 2014).

In contrast, effective cyanobacterial degradation of aliphatic hydrocarbon has been reported for *Aphanothece conferta* and *Synechocystis aquatilis* (Ibraheem 2010). Such results are in agreement with previous reports showing the degradation of *n*-alkanes by *Microcoleus chthonoplastes* and *Phormidium corium* isolated from oil-contaminated sediments (Al-Hasan et al. 1998). Although the cyanobacteria were in non-axenic cultures, it was demonstrated that the fatty acids obtained from the *n*-alkane oxidation were incorporated into lipid characteristic of cyanobacteria (galactolipids and sulfolipids). In the same way, the partial degradation of PAHs has been reported for *Anabaena cylindrica*, *Phormidium faveolarum*, *Oscillatoria* sp. strain JCM, *Agmenellum* quadruplicatum (Cerniglia et al. 1980a, b), *Phormidium tenue* (Kumar et al. 2009), and *Anabaena fertilissima* (Patel et al. 2016). Additionally, the analysis of the

complete genome of *Synechocystis* sp. strain PCC 6803 (http://www.ncbi.nlm.nih.gov) revealed the presence of dioxygenase genes (*rhd*) involved in PAH degradation, suggesting that this cyanobacterium might play a role in the aerobic degradation of aromatic compounds. Raghukumar et al. (2001) showed that *Oscillatoria salina*, *Plectonema terebrans*, and *Aphanocapsa* sp. were able to degrade efficiently crude oil, approximately 45–55% of the total fractions in 10 days. However, their test for the absence of hydrocarbonoclastic bacteria was performed in a peptone-rich medium, which is not suitable for the selection of obligate hydrocarbon-degrading bacteria. Similarly, the capacity of cyanobacteria to degrade hydrocarbons is questionable because axenic cyanobacterial cultures are difficult to obtain and the associated bacteria might play a role in degradation of pollutants (Maldonado et al. 2010).

It is likely that in microbial mats, cyanobacteria play a crucial role in determining the fate of petroleum in different ways: (i) some cyanobacteria strains have the capacity to degrade directly hydrocarbon compounds (Cerniglia et al. 1980a, b; Kumar et al. 2009; Patel et al. 2016); (ii) other cyanobacterial strains have an indirect effect in hydrocarbon degradation, immobilizing hydrocarbon-degrading bacteria in their mucilage (Diestra et al. 2005), supplying oxygen and fixed nitrogen to hydrocarbon-degrading bacteria (Ardelean 2014); and (iii) during their growth, cyanobacteria bury oil in deeper anoxic zones where the hydrocarbon degradation relies on anaerobic processes, which is much slower than aerobic degradation. In a culture-based approach, cyanobacterial consortia developed at the interface between the oil and the aqueous culture medium, which in the field would lead to a wrapping effect of the oil layers by these organisms, and so reducing the contact between spilled oil and marine ecosystems (Garcia de Oteyza et al. 2004). The indirect role of cyanobacteria can be important to the overall success of the biodegradation process.

3.2 Role of Other Bacteria in Hydrocarbon Degradation

Aerobic hydrocarbon-degrading bacteria, both generalists and obligate hydrocarbonoclastic bacteria (OHCB), together with anaerobic hydrocarbon-degrading bacteria have been detected and isolated from diverse microbial mats. *Marinobacter*- and *Alcanivorax*-related members were isolated in microbial mats from the Arabian Gulf (Abed et al. 2007). Several strains able to degrade or to grow on hydrocarbons were obtained from polluted microbial mats, including denitrifying bacteria (Goréguès et al. 2004) and sulfate-reducing bacteria (Abed et al. 2011). In addition, members of the *Rhodobacterales* and species related to *Rhodococcus*, *Sphingomonas*, *Xanthomonas*, and *Microbacterium* have been isolated from oil-contaminated microbial mats for their ability to degrade hydrocarbons under oxic conditions (Hernandez-Raquet et al. 2006). All these bacterial groups known to degrade hydrocarbon compounds are frequently detected by molecular approaches in oil-contaminated microbial mats (Aubé et al. 2016; Bordenave et al. 2004a, 2007;

Chronopoulou et al. 2013; Coulon et al. 2012; Cravo-Laureau and Duran 2014; Païssé et al. 2008, 2010; Sanni et al. 2015).

The different functional groups inhabiting microbial mats often work cooperatively, either directly or indirectly, in the degradation of hydrocarbons. Aerobic hydrocarbon-degrading bacteria and cyanobacteria were shown to constitute an ideal model consortium for hydrocarbon biodegradation in which cyanobacteria provide oxygen, fixed nitrogen, and organic compounds to the aerobic degraders (Abed 2010; Abed and Köster 2005; Sánchez et al. 2006). More recently, a synthetic biofilm composed by anoxygenic and oxygenic phototrophic bacteria, diazotrophic bacteria, and hydrocarbon-utilizing bacteria formed a self-sufficient and adequately aerated consortium, showing efficient hydrocarbon biodegradation (Al-Bader et al. 2013). Addition of simple cyanobacterial exudates like glucose and lipids stimulates hydrocarbon biodegradation rates (Radwan et al. 2000), but actual cyanobacterial exudates showed variable effects on bacterial hydrocarbon degradation (Abed 2010). In conclusion, the aerobic hydrocarbon-degrading bacteria in oil-polluted mats constitute a diverse community that contains populations capable of growing on autochthonous (photosynthetic and fermentation products) and allochthonous (alkanes and oil constituents) organic compounds (Abed et al. 2007). The interspecies interactions play a pivotal role in crude oil degradation (McGenity et al. 2012).

As microbial mats develop over the oil, burying it into deeper anoxic zones, anaerobic degradation of petroleum may play a predominant role in oil transformation (Bonin et al. 2004). Simplified communities have been selected under light and anaerobic conditions containing different functional groups, including denitrifiers, sulfate reducers, anoxygenic phototrophs, and fermentative bacteria (Ranchou-Peyruse et al. 2004). The authors demonstrated that denitrifying and sulfate-reducing bacteria performed the active hydrocarbon degradation, whereas other bacteria have an indirect role (e.g., syntrophy, surfactant production). Thus, similar relationship described for cyanobacteria and aerobic hydrocarbon-degrading bacteria might exist between anaerobic anoxygenic phototrophs and heterotrophic anaerobes (denitrifiers, sulfate reducers). For example, several anoxygenic phototrophs, in addition to sulfide reoxidation originating from sulfate reduction, may play an important role in surfactant production (Ranchou-Peyruse et al. 2004; Fig. 3). Nevertheless, in anoxic conditions, the degradation rates are much lower.

In conclusion, microbial mats exhibit strong oil biodegradation potential under both oxic and anoxic conditions as a wide diversity of hydrocarbon-degrading bacteria species inhabit such ecosystems. Under certain conditions, active microbial mats develop on the oil, trapping the oil under anoxic conditions. This phenomenon protects the oil from physical and chemical transformations such as evaporation or photooxidation. The use of microbial mats for in situ bioremediation does not seem to be very effective, because oil degradation is much slower under anoxic conditions than under oxic conditions. However, the relationship existing between the different populations (as affinity of cyanobacteria for oil may facilitate the contact between oil and other potential degrading bacteria) is a promising way of ex situ mitigation processes.

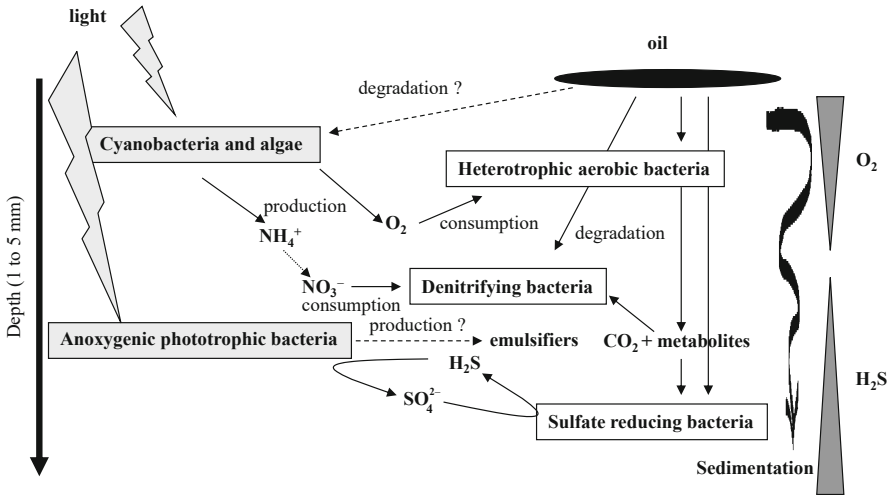

Fig. 3 Hypothetical role of functional bacterial groups inhabiting microbial mats in petroleum compound degradation

4 Research Needs

Since microbial mats develop in coastal sediments, their use for in situ bioremediation processes would constitute a solution for cleaning up oil-polluted coastal sediments. However, oil transformation in microbial mats is often inhibited in situ even if biodegradation potential was demonstrated ex situ. Thus, investigations on how to favor and stimulate the in situ biodegradation mechanisms must be encouraged. For this purpose, the relationship between oil and microbial mats must be further investigated. Oil biodegradation capacities by either pure strains or bacterial consortia or microbial mats are well documented. However, little or nothing is known about how bacterial populations inhabiting microbial mats detect oil, which hydrocarbon-resistance mechanisms are involved, and how the equilibrium between the different bacterial populations is (re)organized when microbial mats are subjected to oil pollution. Thus, further understanding requires not only investigations on their biodegradation capacities but also the development of approaches for the investigation of the whole microbial mat response. Understanding how hydrocarbon pollution may affect the associations between different metabolic populations will provide new insights into the role of microbial mats in oil mitigation in the environment. Systems biology involving omic approaches, such as metagenomics, metatranscriptomics, and metametabolomics is a promising tool to decipher the organization of microbial communities inhabiting microbial mats, their interactions, and their functional diversity involved in hydrocarbon degradation. The challenge is not only to unveil the interaction between the three domains of life (Bacteria, Archaea, and Eukarya) but also to adopt a holistic point of view considering also the grazing pressure and viral predation.

References

Abed RMM (2010) Interaction between cyanobacteria and aerobic heterotrophic bacteria in the degradation of hydrocarbons. Int Biodeterior Biodegrad 64:58–64. https://doi.org/10.1016/j.ibiod.2009.10.008

Abed RMM, Beer DD (2010) Microsensor techniques to study in situ bacterial metabolic processes in hydrocarbon-polluted marine cyanobacterial mats. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 3655–3669. https://doi.org/10.1007/978-3-540-77587-4_288

Abed RMM, Köster J (2005) The direct role of aerobic heterotrophic bacteria associated with cyanobacteria in the degradation of oil compounds. Int Biodeterior Biodegrad 55:29–37. https://doi.org/10.1016/j.ibiod.2004.07.001

Abed RMM, Safi NMD, Köster J, De Beer D, El-Nahhal Y, Rullkötter J, Garcia-Pichel F (2002) Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds. Appl Environ Microbiol 68:1674–1683. https://doi.org/10.1128/AEM.68.4.1674-1683.2002

Abed RMM, Al-Thukair A, De Beer D (2006) Bacterial diversity of a cyanobacterial mat degrading petroleum compounds at elevated salinities and temperatures. FEMS Microbiol Ecol 57:290–301. https://doi.org/10.1111/j.1574-6941.2006.00113.x

Abed RMM, Zein B, Al-Thukair A, de Beer D (2007) Phylogenetic diversity and activity of aerobic heterotrophic bacteria from a hypersaline oil-polluted microbial mat. Syst Appl Microbiol 30:319–330. https://doi.org/10.1016/j.syapm.2006.09.001

Abed RMM, Musat N, Musat F, Mußmann M (2011) Structure of microbial communities and hydrocarbon-dependent sulfate reduction in the anoxic layer of a polluted microbial mat. Mar Pollut Bull 62:539–546. https://doi.org/10.1016/j.marpolbul.2010.11.030

Abed RMM, Al-Kharusi S, Prigent S, Headley T (2014) Diversity, distribution and hydrocarbon biodegradation capabilities of microbial communities in oil-contaminated cyanobacterial mats from a constructed wetland. PLoS One 9. https://doi.org/10.1371/journal.pone.0114570

Akyon B, Stachler E, Wei N, Bibby K (2015) Microbial mats as a biological treatment approach for saline wastewaters: the case of produced water from hydraulic fracturing. Environ Sci Technol 49:6172–6180. https://doi.org/10.1021/es505142t

Al-Bader D, Kansour MK, Rayan R, Radwan SS (2013) Biofilm comprising phototrophic, diazotrophic, and hydrocarbon-utilizing bacteria: a promising consortium in the bioremediation of aquatic hydrocarbon pollutants. Environ Sci Pollut Res 20:3252–3262. https://doi.org/10.1007/s11356-012-1251-z

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. https://doi.org/10.1007/s002270050272

Al-Mailem DM, Sorkhoh NA, Salamah S, Eliyas M, Radwan SS (2010) Oil-bioremediation potential of Arabian Gulf mud flats rich in diazotrophic hydrocarbon-utilizing bacteria. Int Biodeterior Biodegrad 64:218–225. https://doi.org/10.1016/j.ibiod.2010.01.007

Ardelean II (2014) The involvement of cyanobacteria in petroleum hydrocarbons degradation: fundamentals, applications and perspectives. In: Cyanobacteria: ecological importance, biotechnological uses and risk management, pp 41–60

Armitage DW, Gallagher KL, Youngblut ND, Buckley DH, Zinder SH (2012) Millimeter-scale patterns of phylogenetic and trait diversity in a salt marsh microbial mat. Front Microbiol 3. https://doi.org/10.3389/fmicb.2012.00293

Aubé J, Senin P, Pringault O, Bonin P, Deflandre B, Bouchez O, Bru N, Biritxinaga-Etchart E, Klopp C, Guyoneaud R, Goñi-Urriza M (2016) The impact of long-term hydrocarbon exposure on the structure, activity, and biogeochemical functioning of microbial mats. Mar Pollut Bull 111:115–125. https://doi.org/10.1016/j.marpolbul.2016.07.023

Barth HJ (2002) The coastal ecosystems of the Arabian gulf ten years after the 1991 oil spill. Geogr Rundsch 54:36–42

Barth HJ (2003) The influence of cyanobacteria on oil polluted intertidal soils at the Saudi Arabian Gulf shores. Mar Pollut Bull 46:1245–1252. https://doi.org/10.1016/S0025-326X(03)00374-6

Benthien M, Wieland A, García de Oteyza T, Grimalt JO, Kühl M (2004) Oil-contamination effects on a hypersaline microbial mat community (Camargue, France) as studied with microsensors and geochemical analysis. Ophelia 58:135–150

Berthe-Corti L, Nachtkamp M (2010) Bacterial communities in hydrocarbon-contaminated marine coastal environments. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2349–2359. https://doi.org/10.1007/978-3-540-77587-4_171

Bolhuis H, Stal LJ (2011) Analysis of bacterial and archaeal diversity in coastal microbial mats using massive parallel 16S rRNA gene tag sequencing. ISME J 5:1701–1712. https://doi.org/10.1038/ismej.2011.52

Bolhuis H, Cretoiu MS, Stal LJ (2014) Molecular ecology of microbial mats FEMS. Microb Ecol 90:335–350. https://doi.org/10.1111/1574-6941.12408

Bonin P, Cravo-Laureau C, Michotey V, Hirschler-Réa A (2004) The anaerobic hydrocarbon biodegrading bacteria: an overview. Ophelia 58:243–254

Bordenave S, Fourçans A, Blanchard S, Goñi MS, Caumette P, Duran R (2004a) Structure and functional analyses of bacterial communities changes in microbial mats following petroleum exposure. Ophelia 58:195–203

Bordenave S, Jézéque R, Fourçans A, Budzinski H, Merlin FX, Fourel T, Goñi-Urriza M, Guyoneaud R, Grimaud R, Caumette P, Duran R (2004b) Degradation of the "Erika" oil. Aquat Living Resour 17:261–267. https://doi.org/10.1051/alr:2004027

Bordenave S, Goñi-Urriza MS, Caumette P, Duran R (2007) Effects of heavy fuel oil on the bacterial community structure of a pristine microbial mat. Appl Environ Microbiol 73:6089–6097. https://doi.org/10.1128/AEM.01352-07

Bordenave S, Goñi-Urriza M, Vilette C, Blanchard S, Caumette P, Duran R (2008) Diversity of ring-hydroxylating dioxygenases in pristine and oil contaminated microbial mats at genomic and transcriptomic levels. Environ Microbiol 10:3201–3211. https://doi.org/10.1111/j.1462-2920.2008.01707.x

Burow LC, Woebken D, Marshall IPG, Lindquist EA, Bebout BM, Prufert-Bebout L, Hoehler TM, Tringe SG, Pett-Ridge J, Weber PK, Spormann AM, Singer SW (2013) Anoxic carbon flux in photosynthetic microbial mats as revealed by metatranscriptomics. ISME J 7:817–829. https://doi.org/10.1038/ismej.2012.150

Cardoso DC, Sandionigi A, Cretoiu MS, Casiraghi M, Stal L, Bolhuis H (2017) Comparison of the active and resident community of a coastal microbial mat. Sci Rep 7. https://doi.org/10.1038/s41598-017-03095-z

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

Chaillan F, Gugger M, Saliot A, Couté A, Oudot J (2006) Role of cyanobacteria in the biodegradation of crude oil by a tropical cyanobacterial mat. Chemosphere 62:1574–1582. https://doi.org/10.1016/j.chemosphere.2005.06.050

Chronopoulou P-M, Fahy A, Coulon F, Païssé S, Goni-Urriza M, Peperzak L, Acuña-Alvarez L, McKew BA, Lawson T, Timmis KN, Duran R, Underwood GJC, McGenity TJ (2013) Impact of a simulated oil spill on benthic phototrophs and nitrogen-fixing bacteria in mudflat mesocosms. Environ Microbiol 15:242–252. https://doi.org/10.1111/j.1462-2920.2012.02864.x

Cohen Y (2002) Bioremediation of oil by marine microbial mats. Int Microbiol 5:189–193. https://doi.org/10.1007/s10123-002-0089-5

Coulon F, Chronopoulou P-M, Fahy A, Païssé S, Goni-Urriza M, Peperzak L, Acuña-Alvarez L, McKew BA, Brussaard CPD, Underwood GJC, Timmis KN, Duran R, McGenity TJ (2012) Central role of dynamic tidal biofilms dominated by aerobic hydrocarbonoclastic bacteria and diatoms in the biodegradation of hydrocarbons in coastal mudflats. Appl Environ Microbiol 78:3638–3648. https://doi.org/10.1128/aem.00072-12

Cravo-Laureau C, Duran R (2014) Marine coastal sediments microbial hydrocarbon degradation processes: contribution of experimental ecology in the omics' era. Front Microbiol 5. https://doi.org/10.3389/fmicb.2014.00039

Desnues C, Michotey VD, Wieland A, Zhizang C, Fourçans A, Duran R, Bonin PC (2007) Seasonal and diel distributions of denitrifying and bacterial communities in a hypersaline microbial mat (Camargue, France). Water Res 41:3407–3419. https://doi.org/10.1016/j.watres.2007.04.018

Diestra E, Solé A, Esteve I (2004) A comparative study of cyanobacterial diversity in polluted and unpolluted microbial mats by means CLSM. Ophelia 58:151–156

Diestra E, Solé A, Martí M, Garcia de Oteyza T, Grimalt JO, Esteve I (2005) Characterization of an oil-degrading microcoleus consortium by means of confocal scanning microscopy, scanning electron microscopy and transmission electron microscopy. Scanning 27:176–180

Duran R, Cravo-Laureau C (2016) Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. FEMS Microbiol Rev 40:814–830. https://doi.org/10.1093/femsre/fuw031

Duran R, Cravo-Laureau C (2017) Protocols for mudflat and algal mat in situ analysis. In: McGenity TJ, Timmis KN, Nogales B (eds) Hydrocarbon and lipid microbiology protocols: field studies. Springer, Berlin, pp 305–317. https://doi.org/10.1007/8623_2014_42

Duran R, Goñi Urriza MS (2010) Impact of pollution on microbial mats. Microbes and communities utilizing hydrocarbons, oils and lipids. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2339–2348

Duran R, Cuny P, Bonin P, Cravo-Laureau C (2015) Microbial ecology of hydrocarbon-polluted coastal sediments. Environ Sci Pollut Res 22:15195–15199. https://doi.org/10.1007/s11356-015-5373-y

Fan H, Bolhuis H, Stal LJ (2015) Denitrification and the denitrifier community in coastal microbial mats. FEMS Microbiol Ecol 91. https://doi.org/10.1093/femsec/fiu033

Fourçans A, Solé A, Diestra E, Ranchou-Peyruse A, Esteve I, Caumette P, Duran R (2006) Vertical migration of phototrophic bacterial populations in a hypersaline microbial mat from Salins-de-Giraud (Camargue, France). FEMS Microbiol Ecol 57:367–377. https://doi.org/10.1111/j.1574-6941.2006.00124.x

Fourçans A, Ranchou-Peyruse A, Caumette P, Duran R (2008) Molecular analysis of the spatio-temporal distribution of sulfate-reducing bacteria (SRB) in Camargue (France) hypersaline microbial mat. Microb Ecol 56:90–100. https://doi.org/10.1007/s00248-007-9327-x

Garcia de Oteyza T, Grimalt JO (2006) GC and GC-MS characterization of crude oil transformation in sediments and microbial mat samples after the 1991 oil spill in the Saudi Arabian Gulf coast. Environ Pollut 139:523–531. https://doi.org/10.1016/j.envpol.2005.05.022

Garcia de Oteyza TG, Grimalt JO, Diestra E, Solé A, Esteve I (2004) Changes in the composition of polar and apolar crude oil fractions under the action of *Microcoleus* consortia. Appl Microbiol Biotechnol 66:226–232. https://doi.org/10.1007/s00253-004-1694-3

Garcia de Oteyza TG, Grimalt JO, Llirós M, Esteve I (2006) Microcosm experiments of oil degradation by microbial mats. Sci Total Environ 357:12–24. https://doi.org/10.1016/j.scitotenv.2005.04.039

Goréguès C, Michotey V, Bonin P (2004) Isolation of hydrocarbonoclastic denitrifying bacteria from Berre microbial mats. Ophelia 58:263–270

Grant J, Gust G (1987) Prediction of coastal sediment stability from photopigment content of mats of purple sulphur bacteria. Nature 330:244–246

Grötzschel S, Köster J, Abed RMM, De Beer D (2002) Degradation of petroleum model compounds immobilized on clay by a hypersaline microbial mat. Biodegradation 13:273–283. https://doi.org/10.1023/A:1021263009377

Grötzschel S, Köster J, De Beer D (2004) Degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) by a hypersaline microbial mat and related functional changes in the mat community. Microb Ecol 48:254–262. https://doi.org/10.1007/s00248-003-2020-9

Guézennec J, Moppert X, Raguénes G, Richert L, Costa B, Simon-Colin C (2011) Microbial mats in French Polynesia and their biotechnological applications. Process Biochem 46:16–22. https://doi.org/10.1016/j.procbio.2010.09.001

Hamilton TL, Bryant DA, Macalady JL (2016) The role of biology in planetary evolution: cyanobacterial primary production in low-oxygen Proterozoic oceans. Environ Microbiol 18:325–340. https://doi.org/10.1111/1462-2920.13118

Harris JK, Caporaso JG, Walker JJW, Spear JR, Gold NJ, Robertson CE, Hugenholtz P, Goodrich J, McDonald D, Knights D, Marshall P, Tufo H, Knight R, Pace NR (2013) Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. ISME J 7:50–60. https://doi.org/10.1038/ismej.2012.79

Hernandez-Raquet G, Budzinski H, Caumette P, Dabert P, Le Ménach K, Muyzer G, Duran R (2006) Molecular diversity studies of bacterial communities of oil polluted microbial mats from the Etang de Berre (France). FEMS Microbiol Ecol 58:550–562. https://doi.org/10.1111/j.1574-6941.2006.00187.x

Ibraheem IBM (2010) Biodegradability of hydrocarbons by cyanobacteria. J Phycol 46:818–824. https://doi.org/10.1111/j.1529-8817.2010.00865.x

Kruschel C, Castenholz RW (1998) The effect of solar UV and visible irradiance on the vertical movements of cyanobacteria in microbial mats of hypersaline waters FEMS. Microb Ecol 27:53–72. https://doi.org/10.1111/j.1574-6941.1998.tb00525.x

Kumar MS, Muralitharan G, Thajuddin N (2009) Screening of a hypersaline cyanobacterium, Phormidium tenue, for the degradation of aromatic hydrocarbons: naphthalene and anthracene. Biotechnol Lett 31:1863–1866. https://doi.org/10.1007/s10529-009-0085-3

Kunin V, Raes J, Harris JK, Spear JR, Walker JJ, Ivanova N, Von Mering C, Bebout BM, Pace NR, Bork P, Hugenholtz P (2008) Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. Mol Syst Biol 4. https://doi.org/10.1038/msb.2008.35

Maldonado J, Diestra E, Huang L, Domènech AM, Villagrasa E, Puyen ZM, Duran R, Esteve I, Solé A (2010) Isolation and identification of a bacterium with high tolerance to lead and copper from a marine microbial mat in Spain. Ann Microbiol 60:113–120. https://doi.org/10.1007/s13213-010-0019-2

McGenity TJ, Folwell BD, McKew BA, Sanni GO (2012) Marine crude-oil biodegradation: a central role for interspecies interactions. Aquat Biosyst 8:10. https://doi.org/10.1186/2046-9063-8-10

Megharaj M, Singleton I, McClure NC, Naidu R (2000) Influence of petroleum hydrocarbon contamination on microalgae and microbial activities in a long-term contaminated soil. Arch Environ Contam Toxicol 38:439–445. https://doi.org/10.1007/s002449910058

Mills HJ, Martinez RJ, Story S, Sobecky PA (2004) Identification of members of the metabolically active microbial populations associated with Beggiatoa species mat communities from Gulf of Mexico cold-seep sediments. Appl Environ Microbiol 70:5447–5458. https://doi.org/10.1128/AEM.70.9.5447-5458.2004

Païssé S, Coulon F, Goñi-Urriza M, Peperzak L, McGenity TJ, Duran R (2008) Structure of bacterial communities along a hydrocarbon contamination gradient in a coastal sediment. FEMS Microbiol Ecol 66:295–305. https://doi.org/10.1111/j.1574-6941.2008.00589.x

Païssé S, Goñi-Urriza M, Coulon F, Duran R (2010) How a bacterial community originating from a contaminated coastal sediment responds to an oil input. Microb Ecol 60:394–405. https://doi.org/10.1007/s00248-010-9721-7

Païssé S, Duran R, Coulon F, Goñi-Urriza M (2011) Are alkane hydroxylase genes (alkB) relevant to assess petroleum bioremediation processes in chronically polluted coastal sediments? Appl Microbiol Biotechnol 92:835–844. https://doi.org/10.1007/s00253-011-3381-5

Païssé S, Goñi-Urriza M, Stadler T, Budzinski H, Duran R (2012) Ring-hydroxylating dioxygenase (RHD) expression in a microbial community during the early response to oil pollution. FEMS Microbiol Ecol 80:77–86. https://doi.org/10.1111/j.1574-6941.2011.01270.x

Patel JG, Kumar JIN, Kumar RN, Khan SR (2016) Biodegradation capability and enzymatic variation of potentially hazardous polycyclic aromatic hydrocarbons – anthracene and pyrene by *Anabaena fertilissima*. Polycycl Aromat Compd 36:72–87. https://doi.org/10.1080/10406638.2015.1039656

Pringault O, Aube J, Bouchez O, Klopp C, Mariette J, Escudie F, Senin P, Goni-Urriza M (2015) Contrasted effects of natural complex mixtures of PAHs and metals on oxygen cycle in a microbial mat. Chemosphere 135:189–201. https://doi.org/10.1016/j.chemosphere.2015.04.037

Radwan SS, Al-Mailem D, El-Nemr I, Salamah S (2000) Enhanced remediation of hydrocarbon contaminated desert soil fertilized with organic carbons. Int Biodeterior Biodegrad 46:129–132. https://doi.org/10.1016/S0964-8305(00)00088-3

Raghukumar C, Vipparty V, David JJ, Chandramohan D (2001) Degradation of crude oil by marine cyanobacteria. Appl Microbiol Biotechnol 57:433–436. https://doi.org/10.1007/s002530100784

Ranchou-Peyruse A, Moppert X, Hourcade E, Hernandez G, Caumette P, Guyoneaud R (2004) Characterization of brackish anaerobic bacteria involved in hydrocarbon degradation: a combination of molecular and culture-based approaches. Ophelia 58:255–262

Roeselers G, Loosdrecht MCMV, Muyzer G (2008) Phototrophic biofilms and their potential applications. J Appl Phycol 20:227–235. https://doi.org/10.1007/s10811-007-9223-2

Sánchez O, Ferrera I, Vigués N, García de Oteyza T, Grimalt J, Mas J (2006) Role of cyanobacteria in oil biodegradation by microbial mats. Int Biodeterior Biodegrad 58:186–195. https://doi.org/10.1016/j.ibiod.2006.06.004

Sanni GO, Coulon F, McGenity TJ (2015) Dynamics and distribution of bacterial and archaeal communities in oil-contaminated temperate coastal mudflat mesocosms. Environ Sci Pollut Res 22:15230–15247. https://doi.org/10.1007/s11356-015-4313-1

Stal LJ, van Gemerden H, Krumbein WE (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiol Lett 31:111–125. https://doi.org/10.1016/0378-1097(85)90007-2

Teske A, Callaghan AV, LaRowe DE (2014) Biosphere frontiers of subsurface life in the sedimented hydrothermal system of Guaymas Basin. Front Microbiol 5. https://doi.org/10.3389/fmicb.2014.00362

Van Bleijswijk J, Muyzer G (2004) Genetic diversity of oxygenic phototrophs in microbial mats exposed to different levels of oil pollution. Ophelia 58:157–164

Van Gemerden H (1993) Microbial mats: a joint venture. Mar Geol 113:3–25. https://doi.org/10.1016/0025-3227(93)90146-M

Wieland A, Kühl M, McGowan L, Fourçans A, Duran R, Caumette P, García de Oteyza T, Grimalt JO, Solé A, Diestra E, Esteve I, Herbert RA (2003) Microbial mats on the Orkney Islands revisited: microenvironment and microbial community composition. Microb Ecol 46:371–390. https://doi.org/10.1007/s00248-002-0108-2

Phototroph-Heterotroph Oil-Degrading Partnerships

3

Raeid M. M. Abed

Contents

Abstract

Contamination of sediments with crude oil promotes the growth of different species of phototrophs and heterotrophs. The coexistence of these two groups in oil-polluted sites suggested a possible contribution to hydrocarbon degradation; however, the exact role and interactions between phototrophs and heterotrophs in the degradation processes have only been recently evaluated. Experiments with axenic and non-axenic cultures of phototrophs suggested that they were unable to degrade hydrocarbons and degradation was mostly attributed to their associated heterotrophs. As primary producers, phototrophs do not have an innate potential for hydrocarbon degradation but can be engaged in symbiosis with oil-degrading heterotrophs. The degradation of hydrocarbons by the associated aerobic heterotrophs can promote the growth of phototrophs by reducing the concentrations of potentially toxic hydrocarbons around them, regenerating CO_2 for photosynthesis, providing metabolites, and reducing the high toxic concentrations of O_2. On the other hand, phototrophs can support the activity of

R. M. M. Abed (✉)
College of Science, Biology Department, Sultan Qaboos University, Muscat, Sultanate of Oman
e-mail: rabed@mpi-bremen.de

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_15

oil-degrading heterotrophs by immobilizing them in their sheaths, providing them with O_2 and fixed nitrogen (in the case of cyanobacteria), and directly supplying them with necessary organics produced by their photosynthetic and fermentation activities. It is concluded that phototrophs and heterotrophs constitute ideal consortia that are efficient in the decontamination of oil-polluted sites, compared to individual organisms. However, further research is required to find out the species specificity and the chemical substances that govern the exact relationship and interaction between phototrophs and heterotrophs.

1 Introduction

The relationships between phototrophs and associated heterotrophs can be competitive for scarce nutrients and other resources or symbiotic stimulating the survival and persistence of each other (Paerl et al. 1993). Both groups coexist in the topmost layer of sediments and microbial mats, as well as in the water column, where active carbon and O_2 cycling takes place (Wieland and Kühl 2006). The photosynthetic activity of phototrophs in such sediments is tightly coupled with the respiration activities of aerobic heterotrophs (Abed et al. 2007; Wieland and Kühl 2006). When a sediment is exposed to oil pollution, the growth of hydrocarbon-degrading chemotrophic bacteria as the key players in natural oil remediation is enormously stimulated. In contrast, the toxicity of oil components has dramatic impacts on indigenous species of eukaryotic microalgae (O'Brien and Dixon 1976; Vandermeulen and Ahem 1976; Batterton et al. 1978; Megharaj et al. 2000) and cyanobacteria (Narro 1987; Abed et al. 2002; Benthien et al. 2004), mainly due to the toxicity of oil components. Nevertheless, oil may favor the development of certain, apparently oil-adapted phototrophs into mat-like structures (Hoffmann 1996; Coulon et al. 2012; Chronopoulou et al. 2013), most likely by inhibiting bioturbating and grazing sediment fauna (Barth 2003; Chronopoulou et al. 2013). Since oil spreads horizontally in aqueous environments, it forms compact long-lasting layers that usually solidify by evaporation of the volatile hydrocarbons and merging with sediment particles. Such layers are expected to favor the growth of anaerobic heterotrophs, mainly sulfate-reducing bacteria, by impeding the transport of oxic water and providing electron donors, mostly hydrocarbons (Abed et al. 2011). The interaction between phototrophs and heterotrophs in oil-polluted sediments seems to contribute significantly to the overall degradation of hydrocarbons.

Phototrophs, including cyanobacteria and eukaryotic algae, are frequently encountered in nature in a wide range of ecosystems, and their growth is often associated with bacteria (Ramanan et al. 2016). The ubiquity of phototrophs in oil-polluted sites and the evidence of their growth on different hydrocarbons gave credence to the belief that they are capable of degrading petroleum compounds. The associated bacteria have been viewed as insignificant, and their contribution to the degradation of hydrocarbons has been often ignored. Research on phototrophic mats and monoclonal cultures of phototrophs has demonstrated a vital role

of heterotrophic bacteria in the degradation of oil contaminants (Abed and Köster 2005; Chaillan et al. 2006; Sanchez et al. 2006; Abed 2010). Nevertheless, phototrophs were deemed essential for the degradation process, and degradation rates were higher in their presence (Abed et al. 2002). Hence, phototrophs and associated heterotrophs constitute an ideal consortium for the degradation of hydrocarbons (McGenity et al. 2012). This partnership has been the focus of extensive research over the past decades to find out the exact role of each group of microorganisms in hydrocarbon degradation processes.

2 Phototrophs and Hydrocarbon Degradation

Although phototrophs are capable of producing organics via oxygenic photosynthesis and autotrophy, some strains possessed the ability to grow heterotrophically or photoheterotrophically on organic compounds (Radwan and Al-Hasan 2000). Most of these organics were sugars, but growth on aliphatic and aromatic hydrocarbons has also been reported (Radwan and Al-Hasan 2000; Prince 2018). Several studies on the degradation of a wide range of hydrocarbons by different phototrophic strains have been published (for details see Radwan and Al-Hasan 2000). Molecular studies have also detected catabolic genes, such as monooxygenase and dioxygenase systems, for catalyzing the breaking of the aromatic ring, in several strains of phototrophs (Vidyashankar and Ravishankar 2016). Early studies reported on the degradation of motor oil and crude oil by the alga *Prototheca zopfii* (Walker et al. 1975) and phenol and catechol degradation by several strains of microalgae including *Chlamydomonas ulvaensis*, *Chlorella pyrenoidosa*, and *Scenedesmus brasiliensis* (Ellis 1977). The diatoms *Navicula* sp. and *Nitzschia linearis* could degrade a naphthenic acid and different *n*-alkanes and aromatics, respectively (Gamila and Ibrahim 2004; Headley et al. 2008). The cyanobacterial strains *Oscillatoria* sp. and *Agmenellum quadruplicatum* have been shown to oxidize naphthalene to *cis*-1,2-dihydroxy-1,2-dihydronaphthalene, 4-hydroxy-1-tetralone, and 1-naphthol (Cerniglia et al. 1980a, b), while the latter cyanobacterium degraded phenanthrene with a monooxygenase system (Narro 1985). *Oscillatoria* sp. strain JCM oxidized biphenyl to 4-hydroxybiphenyl (Cerniglia et al. 1980b). Degradation of *n*-alkanes was also demonstrated by the cyanobacteria *Microcoleus chthonoplastes* and *Phormidium corium* (Al-Hasan et al. 1998). In a particular study, it was even stated that the marine cyanobacteria *Oscillatoria salina*, *Plectonema terebrans*, and *Aphanocapsa* were responsible for the degradation of 45–55% of the total fractions of crude oil, which contained 50% aliphatics, 31% waxes and bitumen, 14% aromatics, and 5% polar compounds within 10 days (Raghukumar et al. 2001).

Despite the overwhelming literature on the degradation of hydrocarbons by phototrophs, rigorous evidence is lacking. In most biodegradation studies with phototrophs, it was not clearly stated whether the strains employed were indeed bacteria-free. It is known to be very difficult, although possible, to cultivate axenic phototrophs, but their growth rates are much slower in the absence of associated

bacteria (Fitzsimons and Smith 1984; Abed 2010). If these aerobic heterotrophs exist in low numbers in the culture, their abundance would dramatically increase in the course of the experiment due to their short generation time. Nevertheless, the abundance of phototroph-associated aerobic heterotrophic bacteria was rarely controlled throughout the degradation experiments. Furthermore, in most of the degradation experiments with phototrophs, the maintenance of appropriate controls to account for the non-biological loss of hydrocarbons via photooxidation or evaporation has been rarely reported. All these reasons raised doubts as to whether phototrophs are the actual hydrocarbon degraders or the observed degradation could be attributed to associated heterotrophs or even to abiotic factors. Furthermore, from the perspective that phototrophs have typically an autotrophic mode of life, with their ability to synthesize organics via photosynthesis, it is unlikely that they have a direct participation in the degradation of hydrocarbons.

3 Heterotrophs Are the Major Oil Degraders

Research over the last three decades has accumulated stronger evidence that phototroph-associated aerobic heterotrophs, and not the phototrophs themselves, were the chief degraders of hydrocarbons (Sorkhoh et al. 1995; Radwan et al. 2002; Abed and Köster 2005). Bacteria identified as *Rhodococcus rhodochrous*, *Arthrobacter nicotianae*, *Pseudomonas* sp., and *Bacillus* sp. were associated with the non-axenic cultures of *Microcoleus chthonoplastes* and *Phormidium corium*. These aerobic heterotrophic bacteria were able to oxidize *n*-alkanes, although the study still suggested a direct role of the cyanobacteria in the degradation process (Al-Hasan et al. 1998). Later on, it was demonstrated that picocyanobacteria from the Arabian Gulf accumulated hydrocarbons in their interthylakoid spaces but without degrading them (Al-Hasan et al. 2001). It was postulated that the degradation of these hydrocarbons was probably carried out by the associated heterotrophic bacteria. Biodegradation experiments with an intact cyanobacterial mat exhibited a similar ability to degrade phenanthrene, dibenzothiophene, pristane, and *n*-octadecane in the dark as in the light, demonstrating a phototroph-independent degradation of these compounds (Abed et al. 2002). The bacterial community after degradation in the dark shifted in the favor of bacteria related to *Holophaga foetida*, *Geothrix fermentans*, and *Chloroflexus aurantiacus*, which were believed to be responsible for the observed degradation. Cyanobacterial isolates from the same mat were obtained but could not degrade any of the tested compounds (Abed and Köster 2005). To rule out the contribution of cyanobacteria in biodegradation, a non-axenic culture of *Oscillatoria* OSC was incubated with alkanes in the dark and/or in the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), which inhibits photosynthesis (Abed and Köster 2005). Alkane degradation was still measurable under these conditions, with a significant increase in the abundance of associated bacteria affiliated with *Gammaproteobacteria* and *Bacteroidetes* (Abed and Köster 2005). A molecular characterization of an oil-degrading culture of *Microcoleus chthonoplastes* revealed the presence of epiphytic bacteria belonging to the genera

Rhizobium, *Agrobacterium*, *Roseobacter*, *Parvibaculum*, and *Pseudoxanthomonas*. Besides the contribution of these associated bacteria to hydrocarbon degradation, it was believed that they also supported the growth of *Microcoleus*, which is known for its inability to fix nitrogen (Sanchez et al. 2005). Another attempt to rule out the role of cyanobacteria in biodegradation was carried out by growing microbial mats in a reactor in the absence of inorganic carbon and under low O_2 tension, to prevent the growth of cyanobacteria on the CO_2 produced by heterotrophic bacteria (Sanchez et al. 2006). The study concluded that cyanobacteria could not degrade hydrocarbons but instead the observed degradation was attributed to the associated *Alpha-* and *Gammaproteobacteria*, *Chlorobi*, and *Firmicutes*. Biodegradation experiments with an axenic culture of *Phormidium animale* revealed its inability to degrade hydrocarbons in the range of C_{13}–C_{35} under photoautotrophic or heterotrophic conditions, although this strain constituted the bulk of biomass of an oil-degrading cyanobacterial mat from Indonesia (Chaillan et al. 2006). Oil-degrading bacteria have also been detected in association with several cultures of macroalgae from the Arabian Gulf. These bacteria belonged to the nocardioforms and *Acinetobacter* and could degrade 64–98% and 38–56% of the added *n*-octadecane and phenanthrene, respectively, in 2 weeks (Radwan et al. 2002). A culture of *Chlorella sorokiniana* formed a consortium with *Pseudomonas migulae*, and both could degrade phenanthrene in bioreactors (Munoz et al. 2003). The construction of artificial consortia by combining the oil-tolerant microalga *Scenedesmus obliquus* with the heterotrophic bacteria *Sphingomonas* GY2B, *Burkholderia cepacia* GS3C, *Pseudomonas* GP3A, and *Pandoraea pnomenusa* GP3B showed an elevated efficiency in degrading various aliphatic and aromatic hydrocarbons (Tang et al. 2010).

4 Heterotrophs and Growth of Phototrophs

If phototrophs are not the major oil degraders, the question remains why are they dominantly encountered in oil-polluted ecosystems?. Early reports showed that phototrophs were the first colonizers of oil-contaminated sediments (Hoffmann 1996; Höpner et al. 1996). Several laboratory experiments clearly demonstrated that oil-rich sediments supported the formation of dense cyanobacterial mats versus thin loose biofilms on oil-free sediments (Sorkhoh et al. 1992; Musat et al. 2004). The growth and detection of phototrophs in oil-contaminated sites suggest that some types, although not directly involved in the degradation process, can tolerate the toxic effects of hydrocarbons (Abed et al. 2002; Abed and Golubic 2009). For instance, *Synechocystis* PCC6803 became the most dominant cyanobacterium in mats after degradation of four petroleum compounds, but this strain exhibited similar growth with and without hexadecane, indicating its tolerance but inability to grow on hexadecane (Abed et al. 2002; Abed 2010). Using molecular and microscopy tools, it was shown that the oil-tolerant thin filamentous cyanobacteria belonging to the genera *Halomicronema* and *Phormidium* replaced the original oil-sensitive *Oscillatoria*-like cyanobacteria, when a cyanobacterial mat was exposed to oil pollution over 8 months period (Abed and Golubic 2009).

The growth stimulation of phototrophs on oil-rich sediments could be attributed to the fact that the viscous oil provides a favorable substratum for their attachment and growth. Phototrophs are known to produce excessive amounts of exopolymeric substances (EPS), in response to oil (Fig. 1), which consolidate the sediment and result in the formation of microbial mats. The growth of cyanobacteria might be triggered by the oil-degrading bacteria, which provide nutrients and locally released CO_2 (Fig. 1). Indeed, the strong relationship between cyanobacteria and aerobic heterotrophic bacteria is evident from the difficulty to obtain axenic cultures of cyanobacteria (Rippka 1988). On the other hand, reports have demonstrated the ability of marine and freshwater cyanobacteria to produce and accumulate hydrocarbons, predominantly C_{15} and C_{17} and branch-chain alkanes (Liu et al. 2013; Lea-Smith et al. 2015). These cyanobacterial alkanes were likely sufficient to sustain and support the growth of hydrocarbon-degrading bacteria.

Cocultivation experiments showed that the growth of the axenic strain *Synechocystis* PCC6803 was clearly stimulated in the presence of a hexadecane-degrading bacteria (i.e., GM41 strain) and this growth increased further when

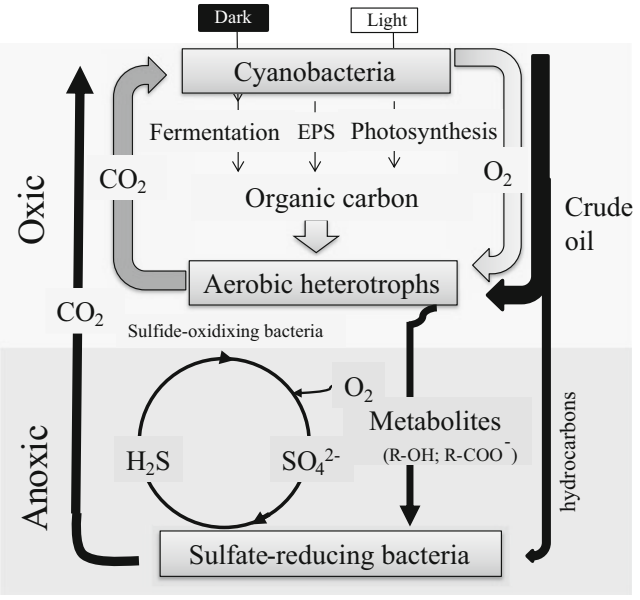

Fig. 1 Scheme depicting the relationship between phototrophs and oil-contaminated marine sediment. Phototrophs (like cyanobacteria) perform photosynthesis during the day producing O_2 and organics and fermentation during the dark producing other types of organics that are taken up by aerobic heterotrophs. Aerobic heterotrophs oxidize a substantial part of the oil to CO_2. Cyanobacteria may benefit from the produced CO_2. Degradation of oil by aerobic heterotrophs may release oxygenated organic products (metabolites), which, along with a fraction of hydrocarbons from the oil, are utilized by sulfate-reducing bacteria (SRB). Produced H_2S is aerobically reoxidized by sulfide-oxidizing bacteria

hexadecane was added to the medium (Abed 2010). Similarly, the presence of alkane-degrading bacteria, capable of degrading black oil, was shown to stimulate the growth of oil-tolerant algal and cyanobacterial species belonging to the genera *Stichococcus*, *Chlorella*, *Scenedesmus*, *Nostoc*, and *Phormidium* (Safonova et al. 1999). Maximum efficiency of oil degradation was achieved when these alkane-degrading bacteria were combined with the phototrophic strains. The alkane-degrading bacteria could even restore the productivity of algae sensitive to black oil and support their growth (Safonova et al. 1999). It is believed that aerobic heterotrophic bacteria support the growth of phototrophs by consuming the organics produced via photosynthesis (Fig. 1), whose accumulation could inhibit photosynthesis (Bateson and Ward 1988). The degradation of hydrocarbons by the aerobic heterotrophs also results in the reduction of the concentrations of these toxic substances around phototrophs (Megharaj et al. 2000). On the other hand, phototrophs could grow heterotrophically on the metabolites (e.g., organic acids and fatty acids) produced during the degradation of hydrocarbons by the aerobic heterotrophic bacteria (Fig. 1) (Radwan and Al-Hasan 2000). The respiration activities of aerobic heterotrophic bacteria counteract the chemical changes in O_2, CO_2, and pH induced by photosynthesis (Whitton 1973; Wieland and Kühl 2006). High O_2 concentrations produced during photosynthesis have an inhibitory effect on RubisCO carboxylase activity and result in photooxidation in phototrophs (Garcia-Pichel et al. 1999). The direct utilization of photosynthetic exudates and/or hydrocarbons by the aerobic heterotrophs (Fig. 1) results in the regeneration of CO_2 required for photosynthesis (Lange 1967; Bauld and Brock 1974; Paerl 1976; Herbst and Overbeck 1978; Cole 1982; Bateson and Ward 1988; Baines and Pace 1991; Wang and Priscu 1994; Epping et al. 1999; Kirkwood et al. 2006; Amin et al. 2012; Kouzuma and Watanabe 2015). Phototrophs and associated aerobic heterotrophic bacteria have been shown to exchange vitamins, other growth factors, as well as nitrogen and carbon sources, leading to enhanced growth of phototrophs (Burkholder 1963; Paerl 1982; Steppe et al. 1996; Amin et al. 2015). Indeed, stable isotope imaging, based on nanometer-scale secondary ion mass spectrometry (NanoSIMS), clearly demonstrated the ability of aerobic heterotrophic bacteria to assimilate carbon and nitrogen fixed by phototrophs like cyanobacteria (Behrens et al. 2008; Pett-Ridge and Weber 2012).

5 Indirect Role of Phototrophs in Biodegradation

Although phototrophs are not playing a major direct role in the degradation of hydrocarbons in oil-polluted ecosystems, they play an important indirect role in supporting the overall degradation process. They trap and immobilize oil-degrading bacteria in their mucilage, hence preventing them from being washed away. Millions of oil-degrading bacteria have been detected in the sheaths of *Microcoleus chthonoplastes* and *Phormidium corium*, which were isolated from an oil-degrading microbial mat (Radwan and Al-Hasan 2000). Phototrophs also supply the oil-degrading bacteria with O_2 produced by photosynthesis (Fig. 1) (Abed et al. 2002; Abed and Köster 2005). The availability of O_2 is a prerequisite

for successful aerobic degradation (Rosenberg et al. 1992). O_2 becomes limited in oil-polluted ecosystems due to the formation of an oil layer that severely hampers its diffusion. Furthermore, O_2 is scavenged by the high activity of oil-degrading bacteria that can grow directly on hydrocarbons or on intermediate products produced during aerobic degradation (Fig. 1) (Belyaev et al. 1982; Nazina et al. 1985). Indeed, microsensor measurements of oil-polluted microbial mats showed a significant stimulation of surface O_2 consumption and sulfate reduction upon oil addition (Musat et al. 2004). Besides their role in sediment consolidation, the produced EPS by phototrophs facilitate oil emulsification, thus increasing its bioavailability and degradation by the heterotrophs (Flemming and Wingender 2001).

Cyanobacteria support oil biodegradation by providing oil-degrading aerobic heterotrophic bacteria with the fixed nitrogen (Musat et al. 2006). Nitrogen is known to be a crucial parameter for biodegradation and is often limited, especially in marine ecosystems and when there is an excess of organic carbon in the form of hydrocarbons (Musat et al. 2006). Very few oil-degrading aerobic heterotrophic bacteria have been reported with the ability to fix N_2 during their growth on hydrocarbons (Chen et al. 1993; Prantera et al. 2002). Amplification of *nifH* genes from oil-polluted sediments revealed that cyanobacteria are the main contributors of fixed N_2, although *nifH* sequences related to heterotrophic bacteria were also detected (Musat et al. 2006; Chronopoulou et al. 2013). These cyanobacteria belonged to heterocystous types in the light but to non-heterocystous types in the dark. *Anabaena* sp. was found to be the major diazotroph in the light in these contaminated sediments (Musat et al. 2006). The detection of *nifH* sequences related to heterotrophic bacteria in these studies suggests that aerobic heterotrophs may also contribute, albeit to a lesser extent, to N_2 fixation in oil-contaminated sediments. Indeed, N_2-fixing aerobic heterotrophic bacteria have been shown to constitute an oil-degrading consortium with *Microcoleus* (Sanchez et al. 2005).

6 Exudates of Phototrophs and Hydrocarbon Biodegradation

With their ability to perform oxygenic photosynthesis during light and fermentation during dark, phototrophs produce different types of organics that can be directly taken up and oxidized by aerobic heterotrophic bacteria to CO_2 (Fig. 1). These exudates include low molecular weight compounds and EPS, composed primarily of proteins, lipids, and nucleic acids (Decho 1990; Flemming and Wingender 2001). Mannitol, arabinose, and glycolate were identified as excretion and photosynthates products (Hellebust 1965; Bateson and Ward 1988). In the dark and under anoxic conditions, cyanobacteria ferment and produce organics such as acetate, propionate, lactate, and ethanol (Nold and Ward 1996; Jonkers and Abed 2003; Anderson et al. 1987; Jørgensen et al. 1992; Stal 1995; Stal and Moezelaar 1997). Previous studies have demonstrated the existence of different populations of aerobic heterotrophic bacteria that are specialized in the consumption of specific cyanobacterial exudates (Jonkers and Abed 2003; Abed et al. 2007).

Several studies have demonstrated the significance of cyanobacterial exudates in the stimulation of hydrocarbon degradation. For instance, glucose and lipids were

shown to enhance degradation of alkanes in polluted soils from Kuwait (Radwan and Al-Hasan 2000; Radwan et al. 2001). Exudates from *Aphanocapsa* and *Pseudoanabaena* spp. were shown to support the growth of a phenol-degrading *Pseudomonas* and a dichloroacetate-degrading *Ancylobacter* (Kirkwood et al. 2006). Nevertheless, these exudates repressed the degradation of phenol by *Pseudomonas* but enhanced the degradation of the dichloroacetate by *Ancylobacter* (Kirkwood et al. 2006). The inhibition of phenol degradation was attributed to a toxic rather than a competitive effect of the exudates. The effect of cyanobacterial exudates on the degradation of phenanthrene by *Sphingomonas* GM2 strain has also been studied (Abed 2010). While alanine and butanol did not affect the degradation rate of phenanthrene, acetate, pyruvate, and glucose exhibited a stimulatory effect. It was postulated that when the strain is provided with phenanthrene and an exudate simultaneously, the strain would grow on the easily assimilated, low molecular weight, cyanobacterial exudate rather than on phenanthrene. The growth on the exudates will lead to a significant increase in the abundance of the degrading bacteria, after which they degrade hydrocarbons. Indeed, protein analysis supported the increase in bacterial biomass by three- to fourfold in the presence of the exudate (Abed 2010). Hydrocarbon-degrading bacteria were also found in high densities in rhizospheres of higher plants, benefiting from the root exudates, which include easily assimilated compounds like sugars and amino acids (Lee et al. 1995; Radwan et al. 1998). Indeed, several successful bioremediation attempts around the world, including the Exxon Valdez oil spill, have employed organic fertilizers that contain fatty acids, alcohols, and sugars to stimulate degradation rates (Rivet et al. 1993; Bragg et al. 1994; Al-Hadhrami et al. 1996).

7 Research Needs

The symbiotic relationship between heterotrophs and phototrophs is beneficial for both groups and supports the degradation of hydrocarbons. However, it is still unknown whether this relationship is always symbiotic or can also be competitive for nutrients and other limited resources. Furthermore, it is also unknown whether this relationship is species-specific and whether different strains of phototrophs have similar or different populations of associated heterotrophs. Although phototrophs and heterotrophs produce substances that govern their relationship, the identity of these chemicals has not been exactly revealed. The production of such chemicals offers a great potential for the discovery and development of new drugs and bioactive compounds that still awaits further research. Future research should utilize transcriptomic, metagenomic, and metabolomic approaches to expand our understanding of the phototroph-heterotroph interactions (Cooper and Smith 2015). Furthermore, research should also focus on how to exploit oil-degrading heterotrophic-phototrophic consortia for bioremediation and to check their applicability in the field. Other biotechnological applications of heterotrophic-phototrophic consortia in wastewater treatment, biofuels, biorefineries, and bloom control should be explored (Subashchandrabose et al. 2011; Ramanan et al. 2016).

References

Abed RMM (2010) Interaction between cyanobacteria and aerobic heterotrophic bacteria in the degradation of hydrocarbons. Int Biodeterior Biodegrad 64:58–64

Abed RMM, Golubic S (2009) Selection of oil-tolerant cyanobacteria on oil polluted sediments. Algol Stud 130:89–99

Abed RMM, Köster J (2005) The direct role of aerobic heterotrophic bacteria associated with cyanobacteria in the degradation of oil compounds. Int Biodeterior Biodegrad 55:29–37

Abed RMM, Safi NMD, Köster J, de Beer D, Rullkötter J, Garcia-Pichel F (2002) Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds. Appl Environ Microbiol 68:1674–1683

Abed RMM, Kohls K, de Beer D (2007) Effect of salinity changes on the bacterial diversity, photosynthesis and oxygen consumption of cyanobacterial mats from an intertidal flat of the Arabian Gulf. Environ Microbiol 9:1384–1392

Abed RMM, Musat N, Musat F, Mußmann M (2011) Structure of microbial communities and hydrocarbon-dependent sulfate reduction in the anoxic layer of a polluted microbial mat. Mar Pollut Bull 62:539–546

Al-Hadhrami MN, Lappin-Scott HM, Fisher PJ (1996) Effects of the addition of organic carbon sources on bacterial respiration and *n*-alkane biodegradation of Omani crude oil. Mar Pollut Bull 32:351–357

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

Al-Hasan RH, Khanfar M, Eliyas M, Radwan SS (2001) Hydrocarbon accumulation by picocyanobacteria from the Arabian Gulf. J Appl Microbiol 91:533–540

Amin SA, Parker MS, Armbrust EV (2012) Interactions between diatoms and bacteria. Microbiol Mol Biol Rev 76(3):667–684

Amin SA, Hmelo LR, van Tol HM, Durham BP, Carlson LT, Heal KR, Morales RL, Berthiaume CT, Parker MS, Djunaedi B, Ingalls AE, Parsek MR, Moran MA, Armbrust EV (2015) Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature 522(7554):98–101

Anderson KL, Tayne TA, Ward DM (1987) Formation and fate of fermentation products in hot-spring cyanobacterial mats. Appl Environ Microbiol 53:2343–2352

Baines SB, Pace ML (1991) The production of dissolved organic matter by phytoplankton and its importance to bacteria: pattern across marine and freshwater systems. Limnol Oceanogr 36:1078–1090

Barth JJ (2003) The influence of cyanobacteria on oil polluted intertidal soils at the Saudi Arabian Gulf shores. Mar Pollut Bull 46:1245–1252

Bateson MM, Ward DM (1988) Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat. Appl Environ Microbiol 54:1738–1743

Batterton J, Winters K, Baalen CV (1978) Sensitivity of three microalgae to crude oils and fuel oils. Mar Environ Res 1:31–41

Bauld J, Brock TD (1974) Algal excretion and bacterial assimilation in hot spring algal mats. J Phycol 10:101–106

Behrens S, Losekann T, Pett-Ridge J, Weber PK, Ng WO, Stevenson BS, Hutcheon ID, Relman DA, Spormann AM (2008) Linking microbial phylogeny to metabolic activity at the single-cell level by using enhanced element labeling-catalyzed reporter deposition fluorescence in situ hybridization (EL-FISH) and NanoSIMS. Appl Environ Microbiol 74:3143–3150

Belyaev SS, Laurinavichus KS, Obratsova SN, Gorlatov SN, Ivanov MV (1982) Microbiological processes in the critical zone of injection wells in oilfields. Microbiol (Transl. from Mikrobiologiya, Moscow) 51:793–797

Benthien M, Wieland A, de Oteyza TG, Grimalt JO, Kühl M (2004) Oil contamination effects on a hypersaline microbial mat community (Camargue, France) as studied with microsensors and geochemical analysis. Ophelia 58:135–150

Bragg GR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 368:413–418

Burkholder PR (1963) Some nutritional relationships among microbes of sea sediments and waters. In: Oppenheimer CH (ed) Symposium on marine microbiology. C.C. Thomas, Springfield, pp 133–150

Cerniglia CE, Baalen CV, Gibson DT (1980a) Metabolism of naphthalene by the cyanobacterium *Oscillatoria* sp., strain JCM. J Gen Microbiol 116:485–494

Cerniglia CE, Gibson DT, Baalen CV (1980b) Oxidation of naphthalene by cyanobacteria and microalgae. J Gen Microbiol 116:495–500

Chaillan F, Gugger M, Saliot A, Coute A, Oudot J (2006) Role of cyanobacteria in biodegradation of crude oil by a tropical cyanobacterial mat. Chemosphere 62:1574–1582

Chen YP, Lopez-de-Victoria G, Lovell CR (1993) Utilization of aromatic compounds as carbon and energy sources during growth and N2-fixation by free living nitrogen fixing bacteria. Arch Microbiol 159:207–212

Chronopoulou P-M, Fahy A, Coulon F, Paissé S, Goñi-Urriza MS, Peperzak L, Acuña Alvarez L, McKew BA, Lawson T, Timmis KN, Duran R, Underwood GJC, McGenity TJ (2013) Impact of a simulated oil spill on benthic phototrophs and nitrogen-fixing bacteria. Environ Microbiol 15:241–252

Cole JJ (1982) Interactions between bacteria and algae in aquatic ecosystems. Annu Rev Ecol Syst 13:291–314

Cooper MB, Smith AG (2015) Exploring mutualistic interactions between microalgae and bacteria in the omic age. Curr Opin Plant Biol 26:147–153

Coulon F, Chronoupolou P-M, Fahy A, Païssé S, Goñi-Urriza MS, Peperzak L, Acuña Alvarez L, McKew BA, Brussard C, Underwood GJC, Timmis KN, Duran R, McGenity TJ (2012) Central role of dynamic tidal biofilms dominated by aerobic hydrocarbonoclastic bacteria and diatoms in the biodegradation of hydrocarbons in coastal mudflats. Appl Environ Microbiol 78:3638–3648

Decho A (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev 28:73–153

Ellis BE (1977) Degradation of phenolic compounds by fresh water algae. Plant Sci Lett 8:213–216

Epping EHG, Khalili A, Thar R (1999) Dynamics of photosynthesis and respiration in an intertidal biofilm. Limnol Oceanogr 44:1936–1948

Fitzsimons AG, Smith RV (1984) The isolation and growth of axenic cultures of planktonic blue-green algae. Br Phycol J 19:156–162

Flemming HC, Wingender J (2001) Relevance of microbial extrapolymeric substances (EPSs)-Part I: structural and ecological aspects. Water Sci Technol 43:1–8

Gamila HA, Ibrahim MBM (2004) Algal bioassay for evaluating the role of algae in bioremediation of crude oil: I-isolated strains. Bull Environ Contam Toxicol 73:883–889

Garcia-Pichel F, Kühl M, Nübel U, Muyzer G (1999) Salinity dependent limitation of photosynthesis and oxygen exchange in microbial mats. J Phycol 35:184–195

Headly JV, Du JL, Peru KM, Gurprasad N, McMartin DW (2008) Evaluation of algal phytodegradation of petroleum naphthenic acids. J Environ Sci Health A 43:227–232

Hellebust J (1965) Excretion of some organic compounds by marine phytoplankton. Limnol Oceanogr 10:192–206

Herbst V, Overbeck J (1978) Metabolic coupling between the alga *Oscillatoria redekei* and accompanying bacteria. Naturwissenschaften 65:598–599

Hoffmann L (1996) Recolonization of the intertidal flats by microbial mats after the Gulf War oil spill. In: Krupp F, Abduzinada AH, Nader IA (eds) A marine wildlife sanctuary for the Arabian Gulf: environmental research and conservation following the 1991 Gulf War oil spill. Senkenberg Research Institute/NCWCD, Riyad/Frankfurt, pp 96–115

Höpner T, Yousef M, Berthe-Corti L, Felzmann H, Struck H, Al-Thukair A (1996) Cyanobacterial mats on oil-polluted sediments–start of a promising self-remediation process? In: Krupp F, Abuzinada AH, Nader IA (eds) A marine wildlife sanctuary for the Arabian Gulf: environmental

research and conservation following the 1991 Gulf War oil spill. NCWCD/Senckenberg Research Institute, Riyadh/Frankfurt, pp 85–95
Jonkers HM, Abed RMM (2003) Identification of aerobic heterotrophic bacteria from the photic zone of a hypersaline microbial mat. Aquat Microb Ecol 30:127–133
Jørgensen BB, Nelson DC, Ward DM (1992) Chemotrophy and decomposition in modern microbial mats. In: Schopf JW, Klein C (eds) The proterozoic biosphere: a multidisciplinary study. Cambridge University Press, Cambridge, pp 287–293
Kirkwood AE, Nalewajko C, Fulthorpe RR (2006) The effects of cyanobacterial exudates on bacterial growth and biodegradation of organic contaminants, Microb Ecol 51:4–12
Kouzuma A, Watanabe K (2015) Exploring the potential of algae-bacteria interactions. COBiotech 33:125–129
Lange W (1967) Effect of carbohydrates on the symbiotic growth of planktonic blue-green algae with bacteria. Nature 215:1277–1278
Lea-Smith DJ, Biller SJ, Davey MP, Cotton CAR, Perez Sepulveda BM, Turchyn AV, Scanlan DJ, Smith AG, Chisholm SW, Howe CJ (2015) Contribution of cyanobacterial alkane production to the ocean hydrocarbon cycle. Proc Natl Acad Sci U S A 112:13591–13596
Lee K, Tremblay GH, Cobanli SE (1995) Bioremediation of oiled beach sediments: assessment of inorganic and organic fertilizers. In: Proceedings of the 1991 oil spill conference. American Petroleum Institute, Washington, DC, pp 107–113
Liu A, Zhu T, Lu X, Song L (2013) Hydrocarbon profiles and phylogenetic analyses of diversified cyanobacterial species. Appl Energy 111:383–393
McGenity TJ, Folwell BD, McKew BA, Sanni GO (2012) Marine crude-oil biodegradation: a central role for interspecies interactions. Aquatic Biosyst 8:10
Megharaj M, Singleton I, McClure NC, Naidu R (2000) Influence of petroleum hydrocarbon contamination on microalgae and microbial activities in a long-term contaminated soil. Arch Environ Contam Toxicol 38:439–445
Munoz R, Guieysse B, Mattiasson B (2003) Phenanthrene biodegradation by an algal bacterium consortium in two phase partitioning bioreactors. Appl Microbiol Biotechnol 61:261–267
Musat F, Wieland A, Widdel F (2004) Marine sediment with surface contamination by oil in microcosms for microbiological studies. Ophelia 58:217–222
Musat F, Harder J, Widdel F (2006) Study of nitrogen fixation in microbial communities of oil-contaminated marine sediment microcosms. Environ Microbiol 8:1834–1843
Narro ML (1985) Oxidation of aromatic hydrocarbons by marine cyanobacteria. PhD thesis, The university of Texas at Austin, Austin
Narro ML (1987) Petroleum toxicity and the oxidation of aromatic hydrocarbons. In: Fay P, Baalen CV (eds) The cyanobacteria. Elsevier, Amsterdam, pp 493–511
Nazina TN, Rozanova EP, Kuznetsov SI (1985) Microbial oil transformation processes accompanied by methane and hydrogen sulfide formation. Geomicrobiol J 4:103–130
Nold SC, Ward DM (1996) Photosynthate partitioning and fermentation in hot spring microbial mat communities. Appl Environ Microbiol 62:4598–4607
O'Brien PY, Dixon PS (1976) The effects of oil and oil components on algae: a review. Br Phycol J 11:115–142
Paerl HW (1976) Specific association of the blue-green algae *Anabaena* and *Aphanizomenon* with bacteria in freshwater bloom. J Phycol 12:431–435
Paerl HW (1982) Interactions with bacteria. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Botanical monographs. Blackwell, Oxford, pp 241–462
Paerl HW, Bebout BM, Joye SB, Des Marais DJ (1993) Microscale characterization of dissolved organic-matter production and uptake in marine microbial mat communities. Limnol Oceanogr 38:1150–1161
Pett-Ridge J, Weber PK (2012) NanoSIP: NanoSIMS applications for microbial ecology. Methods Mol Biol 881:375–408. https://doi.org/10.1007/978-1-61779-827-6_13
Prantera MT, Drozdowicz A, Leite SG, Rosado AS (2002) Degradation of gasoline hydrocarbons by two N2-fixing soil bacteria. Biotechnol Lett 24:85–89

Prince RC (2018) Eukaryotic hydrocarbon degraders. In: McGenity TJ (ed) Taxonomy, genomics and ecophysiology of hydrocarbon-degrading microbes. Handbook of hydrocarbon and lipid microbiology. Springer. https://doi.org/10.1007/978-3-319-60053-6_16-1

Radwan SS, Al-Hasan RH (2000) Oil pollution and cyanobacteria. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer, Dordrecht, pp 307–319

Radwan SS, Al-Awandhi H, Sorkhoh NA, El-Nemr IM (1998) Rhizospheric hydrocarbon utilizing microorganisms as potential contributors to phytoremediation for the oily Kuwaiti desert. Microbiol Res 153:247–251

Radwan SS, Al-Aawadi HA, Khanafer M (2001) Effects of lipids on *n*-alkane attenuation in media supporting oil utilizing microorganisms from the oily Arabian Gulf coasts. FEMS Microbiol Lett 198:99–103

Radwan SS, Al-Hasan RH, Salamah S, Al-Dabbous S (2002) Bioremediation of oily sea water by bacteria immobilized in biofilms coating macroalgae. Int Biodeterior Biodegrad 50:55–59

Raghukumar C, Vipparty V, David JJ, Chandramohan D (2001) Degradation of crude oil by marine cyanobacteria. Appl Microbiol Biotechnol 57:433–436

Ramanan R, Kim B-H, Cho D-H, Oh H-M, Kim H-S (2016) Algae-bacteria interactions: evolution, ecology and emerging applications. Biotechnol Adv 34:14–29

Rippka R (1988) Isolation and purification of cyanobacteria. In: Packer L, Glazer AN (eds) Methods in enzymology: cyanobacteria. Academic Press, New York, pp 3–27

Rivet L, Mille G, Basseres A, Ladousse A, Gerin C, Acquaviva M, Bertrand J-C (1993) *n* alkane biodegradation by a marine bacterium in the presence of an oleophilic nutriment. Biotechnol Lett 15:637–640

Rosenberg E, Legmann R, Kushmaro A, Taube R, Adler E, Ron EZ (1992) Petroleum bioremediation – a multiphase problem. Biodegradation 3:337–350

Safonova ET, Dmitrieva IA, Kvitko KV (1999) The interaction of algae with alcanotrophic bacteria in black oil decomposition. Resour Conserv Recycl 27:193–201

Sanchez O, Diestra E, Esteve I, Mas J (2005) Molecular characterization of an oil-degrading cyanobacterial consortium. Microb Ecol 50:580–588

Sanchez O, Ferrera I, Vigues N, de Oteyza TG, Grimalt J, Mas J (2006) Role of cyanobacteria in oil biodegradation by microbial mats. Int Biodeterior Biodegrad 58:186–195

Sorkhoh N, Al-Hasan R, Radwan S (1992) Self-cleaning of the Gulf. Nature 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

Stal LJ (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol 131:1–32

Stal LJ, Moezelaar R (1997) Fermentation in cyanobacteria. FEMS Microbiol Rev 21(179):211

Steppe TF, Olson JB, Paerl HW, Litaker RW, Belnap J (1996) Consortial N_2 fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microbiol Ecol 21:149–156

Subashchandrabose SR, Ramakrishnan B, Megharaj M, Venkatewarlu K, Naidu R (2011) Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. Biotechnol Adv 29:896–907

Tang X, He LY, Tao XQ, Dang Z, Guo CL, Lu GN, Yi XY (2010) Construction of an artificial microalgal-bacterial consortium that efficiently degrades crude oil. J Hazard Mater 181:1158–1162

Vandermeulen JH, Ahem TP (1976) Effect of petroleum hydrocarbons on the algal physiology: review and progress report. In: Lockwood APM (ed) Effects of pollution on aquatic organisms. Cambridge University Press, London, pp 107–125

Vidyashankar S, Ravishankar GA (2016) Algae-based bioremediation: bioproducts and biofuels for biobusiness. In: Prasad MNV (ed) Bioremediation and bioeconomy. Elsevier, Amsterdam, pp 457–483

Walker JD, Colwell RR, Vaituzis Z, Meyer SA (1975) Petroleum-degrading achlorophyllous alga *Prototheca zopfii*. Nature (London) 254:423–424

Wang L, Priscu JC (1994) Stimulation of aquatic bacterial activity by cyanobacteria. Hydrobiologia 277:145–158

Whitton BA (1973) In: Carr NG, Whitton BA (eds) The biology of blue-green algae. 9. Blackwell Scientific, Oxford/London/Edinburg/Melbourne, pp 353–367

Wieland A, Kühl M (2006) Regulation of photosynthesis and oxygen consumption in a hypersaline cyanobacterial mat (Camargue, France) by irradiance, temperature and salinity. FEMS Microbiol Ecol 55:195–210

Microbial Ecology of Marine Environments Chronically Polluted by Petroleum

4

Cristiana Cravo-Laureau and Robert Duran

Contents

Abstract

The marine environment receives continuous input of petroleum hydrocarbons from natural and anthropogenic sources leading to chronically polluted sites. Several studies have demonstrated the adaptation mechanisms of microorganisms to tolerate and/or degrade petroleum hydrocarbons, resulting in altered microbial community composition. This chapter presents information about the microbial assemblages in marine environments chronically contaminated with petroleum and the physical-chemical factors driving their organization. Microbial sentinels are proposed from microbial community studies, drawing on global patterns, as potential indicators of the recovery of the contaminated ecosystems.

1 Introduction

The marine environment is considered as the final receptacle of a wide variety of pollutants resulting in multi-pollution hot spots (Duran et al. 2015a). Petroleum hydrocarbons have been shown to be among the main pollutants entering marine

C. Cravo-Laureau · R. Duran (✉)
Equipe Environnement et Microbiologie, MELODY group, Université de Pau et des Pays de l'Adour, IPREM UMR CNRS 5254, Pau, France
e-mail: Cristiana.cravo-laureau@univ-pau.fr; Robert.duran@univ-pau.fr

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_13

ecosystems, with an annual discharge estimated to be between 1.3 and 8.8 million tons (National Research Council (US) Committee on Oil in the Sea 2003). Although accidental oil spills input spectacular quantities of hydrocarbons in a short time, they represent only a minor share (less than 10%) of the amounts entering the marine environment, the major sources being from natural oil seeps (representing ~47%). The rest comes from anthropogenic sources, principally through fluvial runoff, shipping, offshore mining, and atmospheric deposition (Judd and Hovland 2007). Because coastal areas often have intense human activities, they display numerous chronically polluted sites, especially in estuaries and harbors (Duran et al. 2015c). Poly-aromatic hydrocarbons (PAHs), particularly those with a high molecular weight (HMW), are the most persistent hydrocarbons (Cerniglia 1992). They tend to aggregate and to sorb to particulate and organic matter due to their physical-chemical properties characterized by high hydrophobicity, low solubility, and volatility (Lu et al. 2011). Their persistence in the marine environment is controlled by environmental parameters (Marín-Spiotta et al. 2014), involving biotic and abiotic processes (for a review, see Duran and Cravo-Laureau 2016). PAHs accumulate in sediments, where they are partitioned toward the sorbed phase according to sediment grain size, temperature, and salinity (Frapiccini and Marini 2015). Most hydrocarbons, including PAH, can be biodegraded by the collective catabolic diversity of microorganisms (Head et al. 2006; McGenity et al. 2012; Paissé et al. 2008). The microbial hydrocarbon degradation processes have been extensively described (Kimes et al. 2014; McGenity 2014). However, because the organization of the microbial community structure is influenced by numerous environmental factors, the ecology of microbial communities in marine environments chronically polluted by petroleum hydrocarbons requires further elucidation (Cravo-Laureau and Duran 2014; Duran et al. 2015c). Among the main drivers controlling the organization of microbial community structure in marine sediments are particle grain size and dissolved oxygen concentration (Wang et al. 2013). In this chapter, we review the microbial assemblages described in chronically petroleum-contaminated marine environments and the physical-chemical factors driving their organization. We highlight the importance of performing studies at different levels of biological organization in order to improve our understanding of microbial ecology of marine environments that are chronically contaminated with petroleum.

2 Microbial Assemblages in Marine Environments Chronically Polluted by Petroleum

Contaminated sites that are chronically contaminated by petroleum have been considered as hot spots of hydrocarbon-degrading microorganisms (Bartha 1986). Comparison of the α-diversity from chronically contaminated and nonimpacted sites has shown variable results. Higher microbial diversity has been reported in harbor areas (Zhang et al. 2008), in marinas (Duran et al. 2015a; Nogales et al. 2007), and in coastal sediments near a refinery water treatment plant (Paissé et al. 2008), while reduced diversity has been observed in contaminated beaches (Rosano-Hernández et al. 2012), estuaries (Sun et al. 2012, 2013), coastal sediments near highly industrialized areas (Korlević et al.

2015; Quero et al. 2015), and marine oil seeps (LaMontagne et al. 2004). Anyway, the β-diversity, comparing microbial communities from chronically contaminated sites with those of nonimpacted sites, highlighted the occurrence of specific microbial populations adapted to the presence of petroleum hydrocarbons (Duran et al. 2015a; Korlević et al. 2015; Paissé et al. 2008) and multi-contamination (Misson et al. 2016). Similarly, several studies have demonstrated that the pollution history affects hydrocarbon-degrading microbial community assemblages and their response to a novel oil input (Bordenave et al. 2007; Hernandez-Raquet et al. 2006; Röling et al. 2002; Sauret et al. 2012). Such observations suggest that preadapted microbial communities exhibit high metabolic capabilities for the tolerance/resistance to oil toxicity and the degradation of hydrocarbon mixtures (Rosano-Hernández et al. 2012), providing a higher organization stability to microbial communities inhabiting chronically contaminated sites (Païssé et al. 2010; Sauret et al. 2012). The adaptive features for the tolerance/resistance to hydrocarbons include physiological properties such as a hydrophobic cell envelope associated with active transport systems, efflux pumps, and hydrocarbon degradation pathways as described for *Rhodococcus*-related species (for a review, see de Carvalho et al. 2014). Similarly, the obligate hydrocarbonoclastic bacteria (OHCB; Yakimov et al. 2007), which use hydrocarbon compounds, almost exclusively, as carbon and energy sources, have been found to be particularly well adapted to environments chronically contaminated with petroleum (Duran 2010; Hernandez-Raquet et al. 2006; Rosano-Hernández et al. 2012; Yakimov et al. 2007). Furthermore, several studies have reported the characterization of hydrocarbonoclastic bacteria isolated from chronically polluted sites showing capacities to degrade hydrocarbons under both aerobic (Baruah et al. 2016; Ben Said et al. 2008; Catania et al. 2015; Guermouche M'rassi et al. 2015; Messina et al. 2016; Teramoto et al. 2013) and anaerobic (Goréguès et al. 2004; Hakil et al. 2014; Venkidusamy and Megharaj 2016) conditions. Molecular approaches have revealed that members related to *Bacteroidetes*, *Deltaproteobacteria*, and *Gammaproteobacteria* dominated bacterial community composition in chronically polluted sediments (Ben Said et al. 2010; Paissé et al. 2008; Zhang et al. 2008), particularly pointing out the importance of sulfate-reducing bacterial communities (Paissé et al. 2008; Stauffert et al. 2015a) and nitrate-reducing bacterial communities (Stauffert et al. 2015b) in hydrocarbon degradation processes. Such molecular-based studies also highlighted the importance of microbial interactions (Ben Said et al. 2015; Duran et al. 2015a; Louati et al. 2013). The direct and indirect interactions between microorganisms build a microbial network, playing a pivotal role in hydrocarbon degradation (McGenity et al. 2012). Such networks have been described in chronically polluted sediments showing bacterial interactions, for example, *Actinobacteria*, which revealed a "specialist" *Actinobacteria* group specifically associated with PAHs and toxic metals (Duran et al. 2015a). Similarly, networks showing bacteria/meiofauna interactions (Ben Said et al. 2015; Louati et al. 2013) and benthic eukaryotic communities interactions, including detritus, ciliates, heterotrophic flagellates, metazoans, parasites, and decomposers (Lanzén et al. 2016), have been reported. Metagenomic analyses showed large diversity of alkane monooxygenase *alkB* genes (Guibert et al. 2016) and ring hydroxylating dioxygenase *rhd* genes (Loviso et al. 2015) in subantarctic coastal sediments chronically contaminated with petroleum, revealing the contribution of diverse bacterial

groups to alkane and PAH degradation, respectively. Hydrocarbon biodegradation metabolic networks in marine sediments chronically contaminated with petroleum have been reconstructed based on metagenomic data, revealing a high abundance of genes involved in the initial degradation of hydrocarbons (Bargiela et al. 2015). Such a result is consistent with the rapid biodegradation observed in response to a novel oil input in chronically polluted marine sediments (Sauret et al. 2012). The high abundance and large diversity of genes involved in degradation processes also suggest functional redundancy in hydrocarbon degradation as shown in mesocosm experiments revealing distinct microbial communities according to the treatment applied (Cravo-Laureau and Duran 2014; Stauffert et al. 2013).

The attempts to develop bioremediation processes for sites chronically polluted by petroleum revealed the importance of oxygenation as a main factor for enhancing the hydrocarbon degradation capacities (Duran et al. 2015b; Nikolopoulou and Kalogerakis 2009). Particularly, oxic/anoxic oscillations have been shown as the main drivers for the organization of microbial communities in coastal marine sediments (Cravo-Laureau and Duran 2014; Militon et al. 2015; Vitte et al. 2011, 2013). The influence of physical-chemical factors on microbial assemblages is discussed in the following paragraph.

3 Global Microbial Community Pattern

Describing and understanding the distribution and abundance of species at broad spatial and temporal scales has become a major preoccupation in microbial ecology. The ability to predict the impact of climate change could be considered as one of the major drivers for such biogeographical studies. Thereby, hypotheses evoked in macroecology are increasingly transposed to microbiology. Theories related to "biogeography of traits," such as the "Bergmann's rule" of size-temperature relationship (Bergmann 1847), have been tested. For example, a decrease of cell size of phytoplankton with increasing ocean temperature has been demonstrated, suggesting the direct effect of temperature or mediated by grazing and nutrient supply (Sommer et al. 2017). Abiotic and biotic environmental processes govern spatial and temporal patterns in the distribution and abundance of microorganisms. The biogeography of microorganisms has been examined, particularly in the last decade (Green and Bohannan 2006; Martiny et al. 2006), and will offer insights into the mechanisms that generate and maintain diversity (speciation, extinction, dispersal, and species interactions). Distinct patterns in microbial biogeography have been reported, such as reversed latitudinal diversity gradient (Amend et al. 2010) or bipolar latitude distributions (Amend et al. 2013; Ladau et al. 2013). Ecological changes occur slowly or sporadically and are only apparent and quantifiable through consistent long-term observation. Thus, temporal patterning in marine bacterial distribution and abundance was observed, suggesting general stability of the bacterial community (Fuhrman et al. 2006). Also, the ecosystem functioning and its response may be dependent of the microbial community structure, particularly when a large portion of the community has low levels of functional redundancy (Fuhrman et al. 2006). But

abiotic factors are important regulators of the stability of microbial communities. Environmental parameters, such as temperature, salinity, and nutrient limitation, affect microbial community structure and function in marine environments (Fuhrman 2009; Fuhrman et al. 2006; Gilbert et al. 2012; Tinta et al. 2015; Ward et al. 2017) as well as interaction with other organisms (Fuhrman et al. 2006; Gilbert et al. 2012). Although large variations in the composition of marine microbial communities have been observed over the short term, their composition has been shown to be resilient and stable over the long term (Fuhrman et al. 2015). Such observations suggest that the relationships between microbial community members are driven by diverse factors varying with time (Fuhrman et al. 2015). Thus, integrating environmental parameters (including those related to human activity, such as pollution) and spatial and long-term variation is crucial to predict the ecology of microorganism assemblages in marine environments and extrapolate from individual samples to the world at large.

Despite the fact that over the past few years many studies have focused on the impact of global change on microbial communities, the microbial global pattern of hydrocarbon-polluted environments is rarely studied. In this way, the impact of temperature on bacterial communities from oil-polluted environments has been reported (Bargiela et al. 2015). At lower temperatures, an increase in total bacterial diversity was observed, whereas a negative effect on the presence and abundance of catabolic genes was shown. These data, considering geographically distributed chronically polluted sites, suggest that temperature is a dominant factor regulating biodegradation capacities. Thereby, the authors suggest investigating sediment and water temperature might disclose regular patterns of behavior with predictive values. In order to validate the extent to which such dynamics follow predictable patterns, macroecological studies merged with holistic approaches could be adopted. Toward this goal, Jeanbille et al. (2016b) investigated the continental and regional pattern of microbial communities in chronically oil-polluted sediments. Using a global approach, the microbial community dynamics and the corresponding environmental parameters were evaluated. Whereas PAHs had no influence on the bacterial and archaeal community structure, the habitat filters, such as salinity, temperature, latitude, and organic carbon content, were characterized as the most important factors driving microbial community diversity. This study also identified eukaryotes as potential biomarkers of chronic contamination, due to their higher sensitivity to PAHs. Nevertheless, the comparison of microbial networks from contaminated areas with those from non-contaminated areas revealed that contamination altered microbial networks (Jeanbille et al. 2016a). Indeed, microbial networks from contaminated areas were less tight, because the interactions between microbial members are affected by the contamination and therefore more vulnerable to environmental perturbations (Jeanbille et al. 2016a).

Searching for pollution biomarkers, many studies have focused on bacteria, suggesting members of *Gammaproteobacteria* as sentinels for the early stages of oil hydrocarbon degradation (Kostka et al. 2011; Yakimov et al. 2007), whereas members of *Alphaproteobacteria* and Gram-positive groups have been proposed as biomarkers for the later stages of degradation (Alonso-Gutierrez et al. 2009; Kasai

et al. 2001; Kostka et al. 2011). These findings arise from consensus observations of a succession of bacterial populations according to the sequential utilization of substrates over time; firstly highly degradable hydrocarbons are degraded, such as aliphatic compounds, followed by more recalcitrant compounds, such as PAHs (Head et al. 2006; McGenity et al. 2012; Rodriguez et al. 2015). Lozada et al. (2014) proposed and tested an ecological index of hydrocarbon exposure based on microbial community composition determined by 16S rRNA gene sequences. They allocated phylotypes according to whether they were from genera that had been shown to include hydrocarbon-degrading species. However, the prediction of the long-term effect on the ecosystem functioning is still extremely difficult to make because generalized patterns with a holistic view are missing (Nogales et al. 2011).

4 Research Requirements

Over the past few decades, much has been learned about the ecological response of microorganisms to oil pollution in marine environments. The ecological succession of microbial communities according to the sequential hydrocarbon degradation, identification of microbial sentinels, resistance, resilience, or functional redundancy of microbial communities could be highlighted as important factors throughout acute and/or chronic pollution (Fig. 1). However, there is a great need to understand

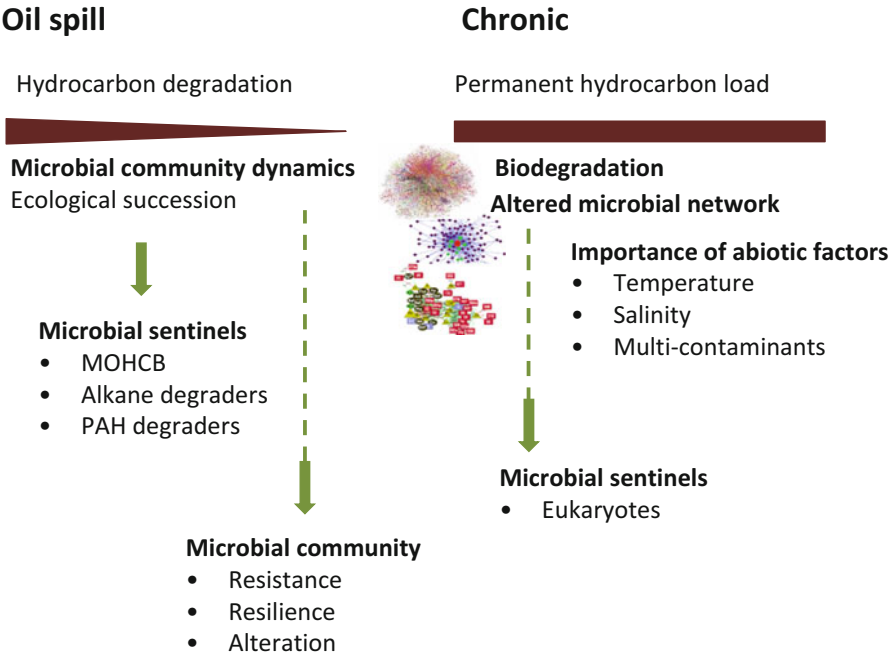

Fig. 1 Key events observed in acute and chronic pollution. *MOHCB* marine obligate hydrocarbonoclastic bacteria, *PAH* poly-aromatic hydrocarbon

microbial interactions, considering, for example, top-down, bottom-up, and sideways controls that influence microbial community structure and function. Also, we have yet to discern how environmental factors interact to regulate the microbial activity in chronically polluted environments.

Spatial and temporal understanding is lacking, which makes it critical to accurately predict the ecology of microbial assemblages and hydrocarbon fate in chronically oil-polluted environments and constrained by climate change. It is thus of paramount importance to determine natural environmental changes at global and long-term scales, not only for a better understanding of perturbations due to human activities but also to gain new insights for a sustainable management of the environment (Joye et al. 2016).

Acknowledgments We thank the support of the French National Research Agency (ANR) for their support through the DECAPAGE (ANR 2011 CESA 006 01) project. We would like to thank all partners of the DECAPAGE project and MELODY group for their useful discussions.

References

Alonso-Gutierrez J, Figueras A, Albaiges J, Jimenez N, Vinas M, Solanas AM, Novoa B (2009) Bacterial communities from shoreline environments (costa da Morte, northwestern Spain) affected by the prestige oil spill. Appl Environ Microbiol 75:3407–3418. https://doi.org/10.1128/aem.01776-08

Amend AS, Seifert KA, Samson R, Bruns TD (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. Proc Natl Acad Sci U S A 107:13748–13753. https://doi.org/10.1073/pnas.1000454107

Amend AS, Oliver TA, Amaral-Zettler LA, Boetius A, Fuhrman JA, Horner-Devine MC, Huse SM, Welch DBM, Martiny AC, Ramette A, Zinger L, Sogin ML, JBH M (2013) Macroecological patterns of marine bacteria on a global scale. J Biogeogr 40:800–811. https://doi.org/10.1111/jbi.12034

Bargiela R, Mapelli F, Rojo D, Chouaia B, Tornés J, Borin S, Richter M, Del Pozo MV, Cappello S, Gertler C, Genovese M, Denaro R, Martínez-Martínez M, Fodelianakis S, Amer RA, Bigazzi D, Han X, Chen J, Chernikova TN, Golyshina OV, Mahjoubi M, Jaouanil A, Benzha F, Magagnini M, Hussein E, Al-Horani F, Cherif A, Blaghen M, Abdel-Fattah YR, Kalogerakis N, Barbas C, Malkawi HI, Golyshin PN, Yakimov MM, Daffonchio D, Ferrer M (2015) Bacterial population and biodegradation potential in chronically crude oil-contaminated marine sediments are strongly linked to temperature. Sci Rep 5:11651. https://doi.org/10.1038/srep11651

Bartha R (1986) Biotechnology of petroleum pollutant biodegradation. Microb Ecol 12:155–172. https://doi.org/10.1007/bf02153231

Baruah R, Kalita DJ, Saikia BK, Gautam A, Singh AK, Deka Boruah HP (2016) Native hydrocarbonoclastic bacteria and hydrocarbon mineralization processes. Int Biodeterior Biodegrad 112:18–30. https://doi.org/10.1016/j.ibiod.2016.04.032

Ben Said O, Goñi-Urriza MS, El Bour M, Dellali M, Aissa P, Duran R (2008) Characterization of aerobic polycyclic aromatic hydrocarbon-degrading bacteria from Bizerte lagoon sediments. Tunis J Appl Microbiol 104:987–997. https://doi.org/10.1111/j.1365-2672.2007.03621.x

Ben Said O, Goñi-Urriza M, Bour ME, Aissa P, Duran R (2010) Bacterial community structure of sediments of the bizerte lagoon (Tunisia), a southern mediterranean coastal anthropized lagoon. Microb Ecol 59:445–456. https://doi.org/10.1007/s00248-009-9585-x

Ben Said O, Louati H, Soltani A, Preud'homme H, Cravo-Laureau C, Got P, Pringault O, Aissa P, Duran R (2015) Changes of benthic bacteria and meiofauna assemblages during bio-treatments

of anthracene-contaminated sediments from Bizerta lagoon (Tunisia). Environ Sci Pollut Res 22:15319–15331. https://doi.org/10.1007/s11356-015-4105-7

Bergmann C (1847) Uber die Verhaltnisse der Warmeokonomie der Thiere zu ihrer Grosse [About the relationship of the thermal economy of animals to their body size.] GottingerStudien 3:538–545

Bordenave S, Goñi-Urriza MS, Caumette P, Duran R (2007) Effects of heavy fuel oil on the bacterial community structure of a pristine microbial mat. Appl Environ Microbiol 73:6089–6097. https://doi.org/10.1128/AEM.01352-07

de Carvalho CCCR, Costa SS, Fernandes P, Couto I, Viveiros M (2014) Membrane transport systems and the biodegradation potential and pathogenicity of genus *Rhodococcus*. Front Physiol 5:133. https://doi.org/10.3389/fphys.2014.00133

Catania V, Santisi S, Signa G, Vizzini S, Mazzola A, Cappello S, Yakimov MM, Quatrini P (2015) Intrinsic bioremediation potential of a chronically polluted marine coastal area. Mar Pollut Bull 99:138–149. https://doi.org/10.1016/j.marpolbul.2015.07.042

Cerniglia C (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3:351–368. https://doi.org/10.1007/BF00129093

Cravo-Laureau C, Duran R (2014) Marine coastal sediments microbial hydrocarbon degradation processes: contribution of experimental ecology in the omics' era. Front Microbiol 5:39. https://doi.org/10.3389/fmicb.2014.00039

Duran R (2010) Marinobacter. In: Timmis K, de Lorenzo V, Van der Meer J-R, TJ MG (eds) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 1725–1735

Duran R, Cravo-Laureau C (2016) Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. FEMS Microbiol Rev 40:814–830. https://doi.org/10.1093/femsre/fuw031

Duran R, Bielen A, Paradžik T, Gassie C, Pustijanac E, Cagnon C, Hamer B, Vujaklija D (2015a) Exploring Actinobacteria assemblages in coastal marine sediments under contrasted human influences in the west Istria Sea, Croatia. Environ Sci Pollut Res 22:15215–15229. https://doi.org/10.1007/s11356-015-4240-1

Duran R, Bonin P, Jezequel R, Dubosc K, Gassie C, Terrisse F, Abella J, Cagnon C, Militon C, Michotey V, Gilbert F, Cuny P, Cravo-Laureau C (2015b) Effect of physical sediments reworking on hydrocarbon degradation and bacterial community structure in marine coastal sediments. Environ Sci Pollut Res 22:15248–15259. https://doi.org/10.1007/s11356-015-4373-2

Duran R, Cuny P, Bonin P, Cravo-Laureau C (2015c) Microbial ecology of hydrocarbon-polluted coastal sediments. Environ Sci Pollut Res 22:15195–15199. https://doi.org/10.1007/s11356-015-5373-y

Frapiccini E, Marini M (2015) Polycyclic aromatic hydrocarbon degradation and sorption parameters in coastal and open-sea sediment. Water Air Soil Pollut 226:246. https://doi.org/10.1007/s11270-015-2510-7

Fuhrman JA (2009) Microbial community structure and its functional implications. Nature 459:193–199

Fuhrman JA, Hewson I, Schwalbach MS, Steele JA, Brown MV, Naeem S (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. Nat Acad Sci U S A 103:13104–13109. https://doi.org/10.1073/pnas.0602399103

Fuhrman JA, Cram JA, Needham DM (2015) Marine microbial community dynamics and their ecological interpretation. Nat Rev Microbiol 13:133–146. https://doi.org/10.1038/nrmicro3417

Gilbert JA, Steele JA, Caporaso JG, Steinbrueck L, Reeder J, Temperton B, Huse S, McHardy AC, Knight R, Joint I, Somerfield P, Fuhrman JA, Field D (2012) Defining seasonal marine microbial community dynamics. ISME J 6:298–308. https://doi.org/10.1038/ismej.2011.107

Goréguès C, Michotey V, Bonin P (2004) Isolation of hydrocarbonoclastic denitrifying bacteria from Berre microbial mats. Ophelia 58:263–270

Green J, Bohannan BJM (2006) Spatial scaling of microbial biodiversity. Trends Ecol Evol 21:501–507. https://doi.org/10.1016/j.tree.2006.06.012

Guermouche M'rassi A, Bensalah F, Gury J, Duran R (2015) Isolation and characterization of different bacterial strains for bioremediation of n-alkanes and polycyclic aromatic hydrocarbons. Environ Sci Pollut Res 22:15332–15346. https://doi.org/10.1007/s11356-015-4343-8

Guibert LM, Loviso CL, Borglin S, Jansson JK, Dionisi HM, Lozada M (2016) Diverse bacterial groups contribute to the alkane degradation potential of chronically polluted subantarctic coastal sediments. Microb Ecol 71:100–112. https://doi.org/10.1007/s00248-015-0698-0

Hakil F, Amin-Ali O, Hirschler-Réa A, Mollex D, Grossi V, Duran R, Matheron R, Cravo-Laureau C (2014) Desulfatiferula berrensis sp. nov., a n-alkene-degrading sulfate-reducing bacterium isolated from estuarine sediments international. J Syst Evol Microbiol 64:540–544. https://doi.org/10.1099/ijs.0.057174-0

Head IM, Jones DM, Roling WFM (2006) Marine microorganisms make a meal of oil. Nat Rev Microbiol 4:173–182

Hernandez-Raquet G, Budzinski H, Caumette P, Dabert P, Le Ménach K, Muyzer G, Duran R (2006) Molecular diversity studies of bacterial communities of oil polluted microbial mats from the Etang de Berre (France). FEMS Microbiol Ecol 58:550–562. https://doi.org/10.1111/j.1574-6941.2006.00187.x

Jeanbille M, Gury J, Duran R, Tronczynski J, Agogué H, Saïd OB, Ghiglione JF, Auguet JC (2016a) Response of core microbial consortia to chronic hydrocarbon contaminations in coastal sediment habitats. Front Microbiol 7:1637. https://doi.org/10.3389/fmicb.2016.01637

Jeanbille M, Gury J, Duran R, Tronczynski J, Ghiglione JF, Agogué H, Saïd OB, Taïb N, Debroas D, Garnier C, Auguet JC (2016b) Chronic polyaromatic hydrocarbon (PAH) contamination is a marginal driver for community diversity and prokaryotic predicted functioning in coastal sediments. Front Microbiol 7:1303. https://doi.org/10.3389/fmicb.2016.01303

Joye SB, Kleindienst S, Gilbert JA, Handley KM, Weisenhorn P, Overholt WA, Kostka JE (2016) Responses of microbial communities to hydrocarbon exposures. Oceanography 29:136–149. https://doi.org/10.5670/oceanog.2016.78

Judd A, Hovland M (2007) Seabed fluid flow: the impact on geology, biology and the marine environment. Cambridge: Cambridge University Press, UK

Kasai Y, Kishira H, Syutsubo K, Harayama S (2001) Molecular detection of marine bacterial populations on beaches contaminated by the Nakhodka tanker oil-spill accident. Environ Microbiol 3:246–255

Kimes NE, Callaghan AV, Suflita JM, Morris PJ (2014) Microbial transformation of the Deepwater horizon oil spill – past, present, and future perspectives. Front Microbiol 5:603. https://doi.org/10.3389/fmicb.2014.00603

Korlević M, Zucko J, Dragić MN, Blažina M, Pustijanac E, Zeljko TV, Gacesa R, Baranasic D, Starcevic A, Diminic J, Long PF, Cullum J, Hranueli D, Orlić S (2015) Bacterial diversity of polluted surface sediments in the northern Adriatic Sea. Syst Appl Microbiol 38:189–197. https://doi.org/10.1016/j.syapm.2015.03.001

Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A, Delgardio J, Norton N, Hazen TC, Huettel M (2011) Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the deepwater horizon oil spill. Appl Environ Microbiol 77:7962–7974. https://doi.org/10.1128/AEM.05402-11

Ladau J, Sharpton TJ, Finucane MM, Jospin G, Kembel SW, O'Dwyer J, Koeppel AF, Green JL, Pollard KS (2013) Global marine bacterial diversity peaks at high latitudes in winter. ISME J 7:1669–1677. https://doi.org/10.1038/ismej.2013.37

LaMontagne MG, Leifer I, Bergmann S, Van De Werfhorst LC, Holden PA (2004) Bacterial diversity in marine hydrocarbon seep sediments. Environ Microbiol 6:799–808. https://doi.org/10.1111/j.1462-2920.2004.00613.x

Lanzén A, Lekang K, Jonassen I, Thompson EM, Troedsson C (2016) High-throughput metabarcoding of eukaryotic diversity for environmental monitoring of offshore oil-drilling activities. Mol Ecol 25:4392–4406. https://doi.org/10.1111/mec.13761

Louati H, Ben Said O, Soltani A, Got P, Mahmoudi E, Cravo-Laureau C, Duran R, Aissa P, Pringault O (2013) The roles of biological interactions and pollutant contamination in shaping microbial benthic community structure. Chemosphere 93:2535–2546. https://doi.org/10.1016/j.chemosphere.2013.09.069

Loviso CL, Lozada M, Guibert LM, Musumeci MA, Sarango Cardenas S, Kuin RV, Marcos MS, Dionisi HM (2015) Metagenomics reveals the high polycyclic aromatic hydrocarbon-degradation

potential of abundant uncultured bacteria from chronically polluted subantarctic and temperate coastal marine environments. J Appl Microbiol 119:411–424. https://doi.org/10.1111/jam.12843

Lozada M, Marcos MS, Commendatore MG, Gil MN, Dionisi HM (2014) The bacterial community structure of hydrocarbon-polluted marine environments as the basis for the definition of an ecological index of hydrocarbon exposure. Microbes Environ 29:269–276. https://doi.org/10.1264/jsme2.ME14028

Lu X-Y, Zhang T, Fang H-P (2011) Bacteria-mediated PAH degradation in soil and sediment. Appl Microbiol Biotechnol 89:1357–1371. https://doi.org/10.1007/s00253-010-3072-7

Marín-Spiotta E, Gruley KE, Crawford J, Atkinson EE, Miesel JR, Greene S, Cardona-Correa C, Spencer RGM (2014) Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries. Biogeochemistry 117:279–297. https://doi.org/10.1007/s10533-013-9949-7

Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach AL, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102–112

McGenity TJ (2014) Hydrocarbon biodegradation in intertidal wetland sediments. Curr Opin Biotechnol 27:46–54. https://doi.org/10.1016/j.copbio.2013.10.010

McGenity TJ, Folwell BD, McKew BA, Sanni GO (2012) Marine crude-oil biodegradation: a central role for interspecies interactions. Aquat Biosyst 8:10. https://doi.org/10.1186/2046-9063-8-10

Messina E, Denaro R, Crisafi F, Smedile F, Cappello S, Genovese M, Genovese L, Giuliano L, Russo D, Ferrer M, Golyshin P, Yakimov MM (2016) Genome sequence of obligate marine polycyclic aromatic hydrocarbons-degrading bacterium Cycloclasticus sp. 78-ME, isolated from petroleum deposits of the sunken tanker Amoco Milford haven, Mediterranean Sea. Mar Genomics 25:11–13. https://doi.org/10.1016/j.margen.2015.10.006

Militon C, Jézéquel R, Gilbert F, Corsellis Y, Sylvi L, Cravo-Laureau C, Duran R, Cuny P (2015) Dynamics of bacterial assemblages and removal of polycyclic aromatic hydrocarbons in oil-contaminated coastal marine sediments subjected to contrasted oxygen regimes. Environ Sci Pollut Res 22:15260–15272. https://doi.org/10.1007/s11356-015-4510-y

Misson B, Garnier C, Lauga B, Dang DH, Ghiglione JF, Mullot JU, Duran R, Pringault O (2016) Chemical multi-contamination drives benthic prokaryotic diversity in the anthropized. Toulon Bay Sci Total Environ 556:319–329. https://doi.org/10.1016/j.scitotenv.2016.02.038

National Research Council (US) Committee on Oil in the Sea (2003). Oil in the Sea III: Inputs, Fates, and Effects. Washington, DC: National Academies Press

Nikolopoulou M, Kalogerakis N (2009) Biostimulation strategies for fresh and chronically polluted marine environments with petroleum hydrocarbons. J Chem Technol Biotechnol 84:802–807. https://doi.org/10.1002/jctb.2182

Nogales B, Aguiló-Ferretjans MM, Martín-Cardona C, Lalucat J, Bosch R (2007) Bacterial diversity, composition and dynamics in and around recreational coastal areas. Environ Microbiol 9:1913–1929. https://doi.org/10.1111/j.1462-2920.2007.01308.x

Nogales B, Lanfranconi MP, Piña-Villalonga JM, Bosch R (2011) Anthropogenic perturbations in marine microbial communities. FEMS Microbiol Rev 35:275–298. https://doi.org/10.1111/j.1574-6976.2010.00248.x

Paissé S, Coulon F, Goñi-Urriza M, Peperzak L, McGenity TJ, Duran R (2008) Structure of bacterial communities along a hydrocarbon contamination gradient in a coastal sediment. FEMS Microbiol Ecol 66:295–305. https://doi.org/10.1111/j.1574-6941.2008.00589.x

Païssé S, Goñi-Urriza M, Coulon F, Duran R (2010) How a bacterial community originating from a contaminated coastal sediment responds to an oil input. Microb Ecol 60:394–405. https://doi.org/10.1007/s00248-010-9721-7

Quero GM, Cassin D, Botter M, Perini L, Luna GM (2015) Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs)

and polychlorinated biphenyls (PCBs). Front Microbiol 6:1053. https://doi.org/10.3389/fmicb.2015.01053

Rodriguez LM, Overholt WA, Hagan C, Huettel M, Kostka JE, Konstantinidis KT (2015) Microbial community successional patterns in beach sands impacted by the Deepwater horizon oil spill. ISME J 9:1928–1940. https://doi.org/10.1038/ismej.2015.5

Röling WFM, Milner MG, Jones DM, Lee K, Daniel F, Swannell RJP, Head IM (2002) Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. Appl Environ Microbiol 68:5537–5548

Rosano-Hernández MC, Ramírez-Saad H, Fernández-Linares L (2012) Petroleum-influenced beach sediments of the Campeche Bank, Mexico: diversity and bacterial community structure assessment. J Environ Manag 95:325–331. https://doi.org/10.1016/j.jenvman.2011.06.046

Sauret C, Christaki U, Moutsaki P, Hatzianestis I, Gogou A, Ghiglione J-F (2012) Influence of pollution history on the response of coastal bacterial and nanoeukaryote communities to crude oil and biostimulation assays. Mar Environ Res 79:70–78. https://doi.org/10.1016/j.marenvres.2012.05.006

Sommer U, Peter KH, Genitsaris S, Moustaka-Gouni M (2017) Do marine phytoplankton follow Bergmann's rule sensu lato? Biol Rev 92:1011–1026. https://doi.org/10.1111/brv.12266

Stauffert M, Cravo-Laureau C, Jézéquel R, Barantal S, Cuny P, Gilbert F, Cagnon C, Militon C, Amouroux D, Mahdaoui F, Bouyssiere B, Stora G, Merlin FX, Duran R (2013) Impact of oil on bacterial community structure in bioturbated sediments. PLoS One 8:e65347. https://doi.org/10.1371/journal.pone.0065347

Stauffert M, Cravo-Laureau C, Duran R (2015a) Dynamic of sulphate-reducing microorganisms in petroleum-contaminated marine sediments inhabited by the polychaete Hediste diversicolor. Environ Sci Pollut Res 22:15273–15284. https://doi.org/10.1007/s11356-014-3624-y

Stauffert M, Cravo-Laureau C, Duran R (2015b) Structure of hydrocarbonoclastic nitrate-reducing bacterial communities in bioturbated coastal marine sediments. FEMS Microbiol Ecol 89:580–593. https://doi.org/10.1111/1574-6941.12359

Sun MY, Dafforn KA, Brown MV, Johnston EL (2012) Bacterial communities are sensitive indicators of contaminant stress. Mar Pollut Bull 64:1029–1038. https://doi.org/10.1016/j.marpolbul.2012.01.035

Sun MY, Dafforn KA, Johnston EL, Brown MV (2013) Core sediment bacteria drive community response to anthropogenic contamination over multiple environmental gradients. Environ Microbiol 15:2517–2531. https://doi.org/10.1111/1462-2920.12133

Teramoto M, Queck SY, Ohnishi K (2013) Specialized hydrocarbonoclastic bacteria prevailing in seawater around a port in the strait of Malacca. PLoS One 8:e66594. https://doi.org/10.1371/journal.pone.0066594

Tinta T, Vojvoda J, Mozetic P, Talaber I, Vodopivec M, Malfatti F, Turk V (2015) Bacterial community shift is induced by dynamic environmental parameters in a changing coastal ecosystem (northern Adriatic, northeastern Mediterranean Sea) – a 2-year time-series study. Environ Microbiol 17:3581–3596. https://doi.org/10.1111/1462-2920.12519

Venkidusamy K, Megharaj M (2016) A novel electrophototrophic bacterium: *Rhodopseudomonas palustris* strain RP2, exhibits hydrocarbonoclastic potential in anaerobic environments. Front Microbiol 7:1071. https://doi.org/10.3389/fmicb.2016.01071

Vitte I, Duran R, Jézéquel R, Caumette P, Cravo-Laureau C (2011) Effect of oxic/anoxic switches on bacterial communities and PAH biodegradation in an oil-contaminated sludge. Environ Sci Pollut Res 18:1022–1032. https://doi.org/10.1007/s11356-010-0435-7

Vitte I, Duran R, Hernandez-Raquet G, Mounier J, Jézéquel R, Bellet V, Balaguer P, Caumette P, Cravo-Laureau C (2013) Dynamics of metabolically active bacterial communities involved in PAH and toxicity elimination from oil-contaminated sludge during anoxic/oxic oscillations. Appl Microbiol Biotechnol 97:4199–4211. https://doi.org/10.1007/s00253-012-4219-5

Wang L, Liu L, Zheng B, Zhu Y, Wang X (2013) Analysis of the bacterial community in the two typical intertidal sediments of Bohai Bay, China by pyrosequencing. Mar Pollut Bull 72:181–187. https://doi.org/10.1016/j.marpolbul.2013.04.005

Ward CS, Yung CM, Davis KM, Blinebry SK, Williams TC, Johnson ZI, Hunt DE (2017) Annual community patterns are driven by seasonal switching between closely related marine bacteria. ISME J 11:1412–1422. https://doi.org/10.1038/ismej.2017.4

Yakimov MM, Timmis KN, Golyshin PN (2007) Obligate oil-degrading marine bacteria. Curr Opin Biotechnol 18:257–266. https://doi.org/10.1016/j.copbio.2007.04.006

Zhang W, Ki J-S, Qian P-Y (2008) Microbial diversity in polluted harbor sediments I: bacterial community assessment based on four clone libraries of 16S rDNA. Estuar Coast Shelf Sci 76:668–681. https://doi.org/10.1016/j.ecss.2007.07.040

Microbial Communities in Hydrocarbon-Polluted Harbors and Marinas

5

Balbina Nogales and Rafael Bosch

Contents

Abstract

Anthropogenically created habitats have characteristic features that determine the composition and function of microbial communities. Harbors are eutrophicated, variable, and complex environments where pollution is chronic and multifactorial (hydrocarbons, heavy metals, biocides, etc.). These environments sustain highly diverse communities, different in composition from those in surrounding areas. Known hydrocarbon degrading bacteria are in low proportions in these communities. The presence of pollutants (hydrocarbons, heavy metals, or combination of both) is an important factor in shaping the composition of these communities.

B. Nogales (✉) · R. Bosch
Departamento de Biología, Universidad de las Illes Balears, Palma, Mallorca, Spain

Instituto Mediterráneo de Estudios Avanzados (IMEDEA, UIB-CSIC), Esporles, Mallorca, Spain
e-mail: bnogales@uib.es; rbosch@uib.es

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_18

But we cannot explain the variability of harbor communities without taking into account multiple and various environmental parameters that might be different for each harbor and even more important than the presence of hydrocarbons. Microbial communities in harbor waters and sediments have capabilities for hydrocarbon degradation and respond rapidly to accidental oil spills in bioremediation trials. We are starting to elucidate the complexity of catabolic networks in harbor communities in terms of microbial taxa and degradation pathways involved, but the data are still scarce. The latest studies show clearly that we should move from the simplistic view that hydrocarbon degradation in harbors is done by well-characterized hydrocarbon degraders. The emerging picture is a network of diverse microorganisms and catabolic activities able to cope with the multiple stress factors acting in harbor environments.

1 Introduction

The marine sector is an important component of world economy. It includes economic activities such as shipbuilding, harbors, transport, fishing, recreational boating, marine equipment, offshore activities, maritime services, and coastal tourism. Maritime transport has grown within the last 30 years in relation to the increase in world gross domestic product, and it constitutes an important fraction of the total trade in the world (maritime trade has been estimated to be over 80% of total world trade (UNCTAD 2016)). The growth of maritime transport goes in parallel with the development of technology as well as the necessary infrastructures (i.e., harbors). Other economically important maritime activities requiring infrastructures are passenger transport, recreational boating, and maritime tourism (Cappato et al. 2011). In the European Union, the value added by the sector of maritime, coastal, and cruise tourism has increased in the last years and represents over 1% of total gross value added (ECORYS 2013).

Harbors are anthropogenically created artificial habitats, with particular features that clearly differentiate them from natural coastal environments. The characteristics of harbors are determined by their location, structure, capacity, hydrology, as well as the activities carried out in them. All harbors are point sources for chronic hydrocarbon pollution derived from boat traffic, accidental spills, discharge of ballast water, and bilge oil. This is accentuated in harbors with facilities for transport of petroleum-derived products. In addition, boat maintenance activities, such as cleaning with detergents, removal of fouling material, painting with antifouling paints, coating or polishing are additional sources of water pollution (i.e., with heavy metals, biocidal and surfactant compounds, nutrients). Since harbors are semi-enclosed water bodies with limited water exchange with the surrounding water masses, their morphology and hydrology are key factors for determining the presence, amount, and residence time of pollutants in these environments

(Gómez et al. 2014; Grifoll et al. 2014; Cutroneo et al. 2017). Impacts tend to be continuous in commercial ports because they are under constant operation. In contrast, the dynamics of recreational marinas is strongly influenced by seasonal factors (i.e., increased traffic in summer months and boat maintenance activities in winter). On the other hand, ports receive also the impact of surrounding industrial, fishing, or urban activities, including discharges of wastewater, in some cases untreated or partially treated. In addition, we have to take into account the atmospheric deposition of hydrocarbons or other pollutants in harbor waters originated by combustion of fossil fuels. This is not only due to operation of the boats themselves but also from engines (i.e., cars, industries) in the surrounding area. Although we can consider that the environmental impact of harbors is inevitable and continuous, the fact is that most harbors and control authorities have protocols to prevent or reduce pollution, as well as for risk assessment (Gómez et al. 2015). For example, harbors and marinas have facilities for the collection of different wastes as addressed, for example, in the International Convention for the Prevention of Pollution from ships (MARPOL convention 73/78 and the following annexes), implemented after the guidance of the International Maritime Organization (IMO) through the Marine Environment Protection Committee (MEPC). This includes regulations for pollution caused by oil, harmful chemicals in bulk or in packaged form, sewage, and garbage as well as control of air pollution (IMO 2003, 2014). Despite all efforts, there might be events of pollution in harbors due to accidents, and these are highly unpredictable in time as well as in the quantity and type of pollutants involved.

Hydrocarbons, particularly those of higher molecular weight, concentrate in harbor sediments from where they can reenter the water column after sediment resuspension, by dredging or natural causes, leading to high concentrations in the water column (King et al. 2004). Losses of hydrocarbons from harbor water due to the weather and especially wind patterns have also been reported (Pérez et al. 2003). The amount of hydrocarbons in harbor waters and sediments is high, sometimes (but not always) exceeding environmental quality standards even by orders of magnitude. The variability in concentrations can also be high, both at the spatial and temporal scales. Some examples of the heterogeneity of polyaromatic hydrocarbons (PAH) concentrations in harbor sediments can be found in recently published studies in samples from Sydney Harbor in Australia (Birch 2017), Brazilian harbors (Pinheiro et al. 2017), Victoria Harbor in Hong-Kong (Chan et al. 2017), San Diego Bay, USA (Neira et al. 2017), and references therein.

The particular characteristics of harbors determine the type of microbial communities in them, and these communities necessarily have to be different from those observed in coastal seawater. The presence of contaminants exerts a selective pressure favoring microorganisms which are either able to degrade the pollutants or to withstand their toxicity. Besides, the inherent heterogeneity and dynamic characteristics of harbors also affects the dynamics of microbial communities. As a result we face a complex system with multiple factors influencing microbial communities.

2 Composition of Microbial Communities in Harbors

2.1 Overall Microbial Diversity in Water and Sediments

In recent years, there has been an increased interest in understanding the composition and functionality of microbial communities in chronically polluted environments. The knowledge of the microbiota from these environments is seen as an opportunity for exploitation (i.e., in pollutant bioremediation) or environmental monitoring (Kalogerakis et al. 2015; Caruso et al. 2016). A number of reports exploring the microbial diversity in harbor water and sediments have been published in the last decade. Most of the diversity studies are based in SSU rDNA analysis, using electrophoretic profiling methods and/or sequencing methods. The harbors studied are in different locations and also differ in size, activity, pollution level, characteristics of the surrounding areas, etc. A particular research effort has been done in Europe, mostly in harbors in the Mediterranean Sea.

Although the intrinsic characteristics of each harbor have an influence on microbial diversity, there are some general findings that define the microbial communities in the harbors studied so far. For example, microbial diversity in these environments is usually high in comparison with unpolluted adjacent coastal areas (Nogales et al. 2007; Zhang et al. 2008; Misson et al. 2016). This seems to contradict the general finding that pollution, with the exception of nutrient enrichment, is associated with a reduction in diversity (Johnston and Roberts 2009). In harbors, these two factors (pollution and nutrients) influence microbial communities and seem to provide conditions to support high diversity. In addition, a heterogeneous spatial distribution of pollutants (e.g., in sediments) contributes to the formation of microzones with varying characteristics that might promote proliferation of diverse microorganisms. The higher organic matter and nutrient content in harbors also sustain higher phytoplankton productivity, as can be seen in the higher concentrations of chlorophyll *a* in harbors. Accordingly, the number of total prokaryotes in harbor waters and sediments is higher than in nearby coastal locations (Schauer et al. 2000; Nogales et al. 2007; Zhang et al. 2007, 2008; Kisand et al. 2012; Barbato et al. 2016; Sauret et al. 2016).

As expected from the meso- to eutrophic characteristics of harbor environments and the presence of multiple pollutants (metals, hydrocarbons, etc.), the microbial communities in harbors are clearly different from those of the adjacent areas. Usually, there is a gradual change in the composition of bacterial communities when approaching the harbors, both in the water and sediment compartments (Nogales et al. 2007; Zhang et al. 2007, 2008; Kisand et al. 2012; Misson et al. 2016; Sauret et al. 2016). In contrast, archaeal communities in sediments do not seem to be so variable at the spatial scale (Misson et al. 2016), although the information available on archaea is very limited. The impact of harbor activities on microbial communities from the surrounding areas seems to be restricted to relatively small scales (1–2 km) (Nogales et al. 2007; Gomes et al. 2013; Sauret et al. 2016). Temporal dynamics of bacterial communities in harbors has been analyzed in the water compartment but not in sediments. In the water, the communities are dynamic and variable at the scale of days (Denaro et al. 2005; Ng et al. 2015) and months

(Nogales et al. 2007). Characteristic bacterial assemblages develop in harbor waters at different seasons such as spring, summer-autumn, and winter (Kan et al. 2006; Nogales et al. 2007; Zhang et al. 2009). This dynamic has been observed in harbors with different geographical location, which might indicate that it has a relation with the seasonal dynamics of marine systems.

The main phyla in harbor waters belong to the domain *Bacteria* and are *Proteobacteria* (classes *Gamma-* and *Alphaproteobacteria*), *Bacteroidetes*, and *Cyanobacteria*. *Actinobacteria* and *Firmicutes* are also found, usually in lower proportion. Most of the sequences retrieved from harbor waters correspond to copiotrophic bacteria, i.e., bacteria responding rapidly to increases in available resources. These include a wide variety of gammaproteobacterial genera (i.e., *Alteromonas*, *Pseudoalteromonas*, *Pseudomonas*, *Colwellia*, *Glaciecola*, *Vibrio*), representatives of the Roseobacter group (i.e., *Phaeobacter*, *Sulfitobacter*), and diverse members of the family *Flavobacteriaceae* (Kan et al. 2006; Zhang et al. 2007, 2009; Nogales et al. 2007; Cappello et al. 2007a, 2012; Aguiló-Ferretjans et al. 2008; Kisand et al. 2012; Ng et al. 2015). In sediments, the main bacterial phylum is also *Proteobacteria*, with three main classes represented: *Gamma-*, *Delta-*, and *Epsilonproteobacteria*. However, there is considerable heterogeneity in the proportion of sequences affiliated to each of these classes. Similar abundances of gamma- and deltaproteobacterial sequences have been reported in several harbors located in Hong Kong, Portugal, and Italy (Zhang et al. 2008; Chiellini et al. 2013; Gomes et al. 2013; Bargiela et al. 2015a). In other harbors (i.e., Milazzo and Messina in Italy, Mucuripe in Brazil), *Deltaproteobacteria* were not detected and most of the sequences belonged to *Gammaproteobacteria* (Yakimov et al. 2005; Bargiela et al. 2015a). Finally, in other cases (Pecém harbor, Brazil), most of the sequences belonged to *Deltaproteobacteria* (Tavares et al. 2016). *Epsilonproteobacteria* were relevant (between 5% and 11%) mainly in Milazzo, Leghorn and Priolo harbors (Yakimov et al. 2005; Chiellini et al. 2013; Bargiela et al. 2015a). There is also considerable heterogeneity in the presence and abundance of other bacterial phyla in harbor sediments. This is the case for *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, or *Planctomycetes* for example. We do not understand the reasons for this heterogeneous distribution of bacterial phyla in harbor sediments. Probably this is due to the combination of different environmental factors and sediment characteristics. On the other hand, all the data from sediments correspond to specific time-point surveys. Without data on population dynamics, we do not know the degree of stability of the communities in harbor sediments. Therefore, we do not know if the observed communities can be considered as representative or characteristic of the different harbors.

2.2 Hydrocarbon-Degrading Microorganisms in Harbor Environments

All studies analyzing the composition of microbial communities in harbor environments had a particular interest in identifying populations likely to be involved in hydrocarbon degradation. Since most studies are based on SSU rDNA sequencing,

authors have inferred the presence of putative hydrocarbon degraders by comparing environmental sequences with those of known hydrocarbon degrading bacteria. Although this is a reasonable approach, the fact is that it has important limitations. Many different microorganisms can degrade hydrocarbons, but this is usually a trait that it is not even species-specific. For example, different strains of the well-known species *Pseudomonas putida* have different catabolic potential (Wu et al. 2011). Therefore, a phylogenetic marker such as SSU rDNA can only give limited information. This approach can be valid to infer the presence of obligate hydrocarbonoclastic bacteria (OHCB) of the Class *Gammaproteobacteria* (Gutierrez 2017), because these bacteria are specialized in using hydrocarbons as a resource for growth (although some of them can use also other substrates such as simple organic acids). However, it cannot be used for genus *Marinobacter* since it contains species that do not degrade hydrocarbons.

The presence of OHCB in harbor waters has been reported only in some studies. For example, in Messina harbor, sequences affiliated to *Alcanivorax*, *Thalassolituus*, *Cycloclasticus*, and *Marinobacter* have been reported occasionally (Denaro et al. 2005; Cappello et al. 2007b). But in most cases, OHCB appear to be under the detection limits of the methodologies employed and therefore should be considered as members of the so-called "rare biosphere" (Sogin et al. 2006). In sediments, OHCB have not been reported or are minor components of the communities (Bargiela et al. 2015a). This is not unexpected since these bacteria are aerobic and sediments tend to be anoxic after few centimeters. In sediments, the inference of putative hydrocarbon degrading microorganisms has a second limitation, i.e., affiliation of taxa is often reported at level of order or family and this resolution is too low. It is generally assumed that some sulfate-reducing bacteria (*Deltaproteobacteria*) detected in sediments might be involved in hydrocarbon degradation due to the level of sequence similarity with degraders or with sequences retrieved from polluted sediments or hydrocarbon seeps (Hayes and Lovley 2002; Zhang et al. 2008; Gomes et al. 2013).

2.3 Environmental Factors Shaping Microbial Communities in Harbors

Some studies have tried to relate the variability in community composition in harbor waters and sediments to environmental parameters. Temperature has emerged as an important factor in shaping bacterial communities in harbors, and it could be one of the reasons of the observed seasonal variability in harbor bacterioplankton (Nogales et al. 2007; Zhang et al. 2009; Sauret et al. 2016). Other relevant factors in the water compartment include salinity (which depends greatly on the presence of freshwater inputs), inorganic nutrients (nitrogen and phosphorus), and chlorophyll *a* which highlights a tight coupling of bacterioplankton with phytoplankton (Nogales et al. 2007; Zhang et al. 2007; Sauret et al. 2016). In Victoria Harbor (Hong Kong), the impact of discharges of treated sewage was evidenced by correlations with parameters indicating eutrophication, such as the concentration of dissolved oxygen or the

biochemical oxygen demand (BOD) and the detection of fecal indicators in the water (Zhang et al. 2009).

The importance of hydrocarbon pollution in shaping the composition of microbial communities in harbors has been analyzed as well. In harbor waters from Marseille area, the concentration of polyaromatic hydrocarbons (PAHs) has been shown to be a factor, although not the most important, in shaping bacterial communities (Sauret et al. 2016). In Messina harbor water, Denaro and collaborators (2005) explained the increase in OHCB by increases in the relative concentration of aliphatic hydrocarbons, a preferred substrate for these bacteria. In sediments, significant correlations with PAHs and other organic pollutants (i.e., polychlorinated biphenyls PCB) have been reported (Duran et al. 2015; Misson et al. 2016). However, these studies have also highlighted the importance of metals (or the combination metals-PAHs) in shaping the diversity and composition of microbial assemblages in harbor sediments (Duran et al. 2015; Barbato et al. 2016; Misson et al. 2016). An interesting approach to address the impact of pollutants in microbial communities of harbor sediments has been the use of co-occurrence network analysis. With this type of analysis, it is possible to find associations between operational taxonomic units (OTUs), as evidence of groups of microorganisms that co-exist in a particular environment and establish relationships to particular environmental conditions (Duran et al. 2015; Jeanbille et al. 2016). Using this approach, Duran and collaborators (2015) could infer three different subnetworks of *Bacteria* and *Actinobacteria* related to the presence of arsenic, toxic metals (Hg, Pb, Cu, Cd) and PAH in harbor sediments, respectively. Since the OTUs in these subnetworks are known, this type of approach can help us to dissect, at higher resolution, the relationships between particular microorganisms and specific pollutants. There is no doubt that this type of information is very valuable for understanding the effect of pollutants on different populations, especially in environments like harbors where we have mixtures of contaminants.

3 Functional Diversity of Harbor Communities

The knowledge of the microbiota in harbors has advanced from mere description of taxa composition to community functionality thanks to the development of *omics* techniques. However, the literature on this topic is still scarce. A comparison between metagenomes of water from Genoa harbor (Italy) and those from unpolluted samples showed differences in the predicted protein function in both environments (Kisand et al. 2012). For example, harbor metagenomes were enriched in categories such as metal resistance genes and efflux pumps, as well as categories related to the central metabolism of aromatic compounds, which might be related to the presence of pollutants. Harbor metagenomes were also enriched in functions related to signal transduction, secretion systems, TonB-like transport systems, cell motility, recombination (integrases and transposases), and plasmid transfer. These are functions characteristic of copiotroph bacteria and communities in which dynamic genetic exchange could be an advantage to adapt to changing conditions (Kisand et al. 2012).

In metagenomes from sediments from Brazilian harbors, sequences related to multidrug efflux pumps and heavy metal resistance mechanisms were observed, in agreement with the presence of metals in the sediment (Tavares et al. 2016). In relation to hydrocarbon pollution, genes for aromatic hydrocarbon degradation (peripheral and central pathways) and for the anaerobic degradation of these compounds were observed. Genes for degradation pathways of nitrotoluene, benzoate, and chlorocyclohexane/chlorobenzoate were predicted in the harbor metagenomes (Tavares et al. 2016).

A study examining sediments that are chronically polluted with hydrocarbons (including two harbors, Priolo and Messina, Italy) combined metagenomics, metaproteomics, and metabolomics to understand the biodegradation potential of these communities (Bargiela et al. 2015a, b). These authors used metagenome data to identify genes encoding enzymes for the catabolism of aromatic compounds, proposed degradation reactions, possible substrates, products, and intermediates of the reactions. This information was used to infer catabolic networks that were experimentally validated with metabolomics data in enrichment experiments. With this approach, the authors demonstrated experimentally the degradation capabilities predicted in the sediment metagenomes (Bargiela et al. 2015a). This is very important because usually assumptions about community functionality are based only on metagenome data, without experimental confirmation. On the other hand, metaproteomics failed to reveal proteins involved in degradation of aromatic compounds in these two harbor sediments (except for a protein involved in protocatechuate degradation). In addition, direct metabolite profiling from harbor sediment samples revealed the accumulation of few compounds (0.5% of total metabolite mass features) that could be associated to pollutants or degradation intermediates (Bargiela et al. 2015b). The authors concluded that this minimal evidence of active biodegradation in the sediments could indicate low degradation rates, probably due to low oxygen availability or the presence of other forms of organic matter (Bargiela et al. 2015b). Alternative hypotheses might be proposed for explaining the apparent low rates of degradation, such as a low bioavailability of the aromatic compounds, or a rapid recycling of degradation metabolites within the microbial community that would lead to low levels of accumulation of metabolites even if the degradation rates are not low. Besides, in the metaproteomes of Priolo and Messina harbor sediments, less than 400 proteins were unambiguously identified, and the authors recognized that this was only a small fraction of the total proteins. These metaproteomes showed clearly the signatures of proteins involved in the metabolism of methane and sulfur compounds, two main functions in anoxic sediments (Bargiela et al. 2015b). However, functions that might be carried out by a smaller fraction of microorganisms (i.e., proteins involved in degradation of pollutants) may remain undetected simply due to resolution of the current metaproteomics methods. In any case, this is an open question because we do not have data on in situ hydrocarbon degradation rates in harbor environments.

4 Exploiting the Microbiota of Harbors for the Degradation of Hydrocarbons

4.1 Water Compartment

During the last decade, several experiments have been done with harbor water samples in order to analyze the changes in the diversity and composition of microbial communities during oil spill bioremediation assays. Even if performed in micro- or mesocosms, these experiments have helped to demonstrate the rapid response of harbor microbiota to sudden oil spills, which are likely to occur in these environments.

A group of scientists in Sicily (Italy) performed several experiments with seawater collected from Messina harbor between years 2003 and 2005 (Cappello et al. 2007a, b, 2012). Two of the experiments were done in 90 L microcosms and a third one at a much bigger scale in a mesocosm of 10,000 L. Overall, they tested the effect of oil addition (Arabian light crude oil) with or without inorganic nutrients (nitrogen and phosphorus) or with a bioemulsifier (EPS_{2003}), as well as the response to inorganic nutrients alone. Despite the fact that the experiments were performed in three consecutive years, there was a common pattern in the response of the microbial communities to the treatments. First of all, the changes in microbial diversity were rapid, already detectable after 2 days of incubation (Cappello et al. 2007a, 2012). Secondly, all treatments changed the composition of the natural microbial communities. In particular, *Alteromonas* sequences, which were in the majority in Messina harbor water samples, decreased or totally disappeared even in the treatment with nutrients alone. This result demonstrated that any perturbation (including nutrient inputs) could lead to changes in diversity. Treatments with oil together with inorganic nutrients or with the bioemulsifier stimulated the growth of hydrocarbonoclastic bacteria (OHCB), mainly *Alcanivorax* and to a lesser extent *Cycloclasticus* and *Oceanospirillum*-related bacteria. In experiments with only oil added, a high proportion of *Pseudomonas*-like sequences were observed after 15 days of incubation (Cappello et al. 2012). Indeed, in all treatments with oil, the authors demonstrated degradation of hydrocarbons, mainly the aliphatic fractions (between 6% and 37% of oil degraded).

In all these experiments, none of the putative hydrocarbon-degrading bacteria detected after the treatments were observed initially in the natural samples with the exception of *Alcanivorax* and *Pseudomonas* in the sample taken from Messina harbor in May 2004 (Cappello et al. 2007b). Therefore, we could expect low hydrocarbon degradation by these bacteria under conditions of normal hydrocarbon concentrations in harbors (i.e., no spill events). The open question is if we understand enough about which microorganisms are playing a role in hydrocarbon degradation under the conditions in harbors (i.e., relatively low-level but long-term hydrocarbon pollution). However, it is clear that hydrocarbon-degrading bacteria find opportunities to proliferate rapidly in these environments in case of a sudden increase in hydrocarbons.

4.2 Sediment Compartment

Due to the anoxic characteristics of submerged sediments after the first few millimeters or centimeters, the main hydrocarbon degradation processes in these environments are anaerobic (Acosta-González and Marqués 2016). Hydrocarbon degradation under sulfate-reducing conditions has been demonstrated in harbor sediments (Coates et al. 1997; Hayes et al. 1999; Rothermich et al. 2002; Dell'Anno et al. 2009, 2012; Rocchetti et al. 2012). This is not unexpected since sulfate reducers, and in particular *Deltaproteobacteria*, are typical degraders in hydrocarbon-polluted anoxic marine environments (Hayes and Lovley 2002; Acosta-González and Marqués 2016).

Exploiting anaerobic hydrocarbon degradation metabolism of autochthonous bacteria appears to be a good strategy for natural attenuation and to reduce the risk associated with hydrocarbons in harbor sediments. Several bioremediation experiments with sediment microcosms from Ancona harbor maintained in anoxic conditions have been published in recent years. These included biostimulation experiments in which sodium acetate or lactose were added as electron donors to enhance sulfate reduction, and bioaugmentation experiments with the addition of sulfate-reducing bacteria (Dell'Anno et al. 2009, 2012; Rocchetti et al. 2012). In all these treatments, the hydrocarbon content of the sediments decreased in comparison with the corresponding controls, supporting the feasibility of such bioremediation approaches. For example, biodegradation yields after 60 days were higher than 40–50% in the treatments and about 24% in the control (Dell'Anno et al. 2009). A limited analysis of the composition of microbial communities was done in one of the studies (Rocchetti et al. 2012). Ribosomal RNA gene sequences affiliated to *Alphaproteobacteria* were dominant in those treatments with lower residual hydrocarbon content, posing a question of their role in hydrocarbon degradation. Gammaproteobacterial OHCB were not detected and sequences from sulfate-reducing *Deltaproteobacteria* (presumed to be the anaerobic degraders) were rare. Therefore, these results did not shed light on the identity of possible hydrocarbon degraders. On the other hand, these experiments also showed that the treatments could affect the bioavailability of different heavy metals in various ways (Dell'Anno et al. 2009; Rocchetti et al. 2012). The results of these studies highlight the importance of the multifactorial character of pollution in harbor sediments and the unknown complexity of interactions between pollutants.

In oxic conditions, there are at least three examples of experimental addition of hydrocarbons and analysis of the response of sediment microbial communities. The first experiment was done in Milazzo harbor sediments testing the effect of inorganic nutrients (nitrogen and phosphorus) alone or together with weathered crude oil, tetradecane, or naphthalene (Yakimov et al. 2005). The other two were done with sediments from Ancona harbor. One of them tested the response of sediment communities to the addition of crude oil and nutrients, including uric acid as an organic nitrogen source (Gertler et al. 2015). The second was an enrichment experiment in ONR7 medium (inorganic nutrients) and different hydrocarbons: crude oil, diesel oil, or naphthalene (Barbato et al. 2016). In all these experiments,

there were changes in microbial communities, which evidenced their dynamics and ability to respond to changes. In contrast with the experiments in anoxic conditions, proliferation of hydrocarbon-degrading bacteria was observed, including the microcosms in which only nutrients were added (Yakimov et al. 2005). This means that providing oxygen and nutrients to sediments was enough to stimulate the growth of these bacteria. An interesting result of these experiments was to show that different putative hydrocarbon degraders proliferated in microcosms with different hydrocarbons. Treatments with oil or diesel oil stimulated growth of *Alcanivorax*, *Marinobacter*, or *Pseudomonas*-like sequences (Yakimov et al. 2005; Barbato et al. 2016). However, with tetradecane, sequences affiliated to *Thalassolituus* and *Oceanospirillum*-like bacteria were predominant. The treatments with naphthalene caused the proliferation of completely different bacterial populations (related to *Neptunomonas/Marinobacterium* and some *Alphaproteobacteria* in the case of Milazzo harbor, and to *Bacillus* and *Halomonas* in Ancona harbor sediments (Yakimov et al. 2005; Barbato et al. 2016). The treatments with uric acid had an effect on the composition of microbial communities and in the proportion of different hydrocarbon-degrading bacteria, favoring, for example, the development of *Marinobacter* (Gertler et al. 2015). Interestingly, there were also differences in the degradation capabilities of the microbial communities developing in treatments with ammonium or uric acid, as well as in catabolic pathway organization at the level of organisms (Bargiela et al. 2015c). This study showed the complexity of the catabolic network in harbor sediments, with bacteria from different taxonomic groups participating in the degradation of the same aromatic compounds (Bargiela et al. 2015c).

All the experiments performed in aerobic microcosms showed the proliferation of hydrocarbon-degrading bacteria. Therefore, stimulation of aerobic biodegradation might be an alternative for hydrocarbon bioremediation in harbor sediments. However, this approach will depend on how efficient is the supply of oxygen (or air) to the sediment. An interesting bioremediation experiment was done in Messina harbor to evaluate the in situ treatment of a simulated oil spill in sediments under aeration conditions (Genovese et al. 2014). In situ aeration of sediments was done using a modular slurry system (MSS). A mesocosm was established with harbor sediments polluted with fuel oil and circulating harbor water. A MSS reactor was inserted inside the mesocosm and air was pumped to the sediment within the reactor. The aim was to stimulate indigenous aerobic hydrocarbon-degrading bacteria and increase biodegradation in the sediments. These authors succeeded in maintaining a positive reduction potential (Eh) in the aerated sediments, in contrast with the anoxic sediments outside the reactor which were highly reduced. Degradation was enhanced in the aerated sediments after 3 months of treatment, with a reduction over 97% of the fuel oil added in comparison with approximately 20% in the anoxic sediments. In anoxic sediments, most of the metabolically active bacteria at the end of the experiment affiliated with *Deltaproteobacteria*. In particular, most of the sequences were related to deltaproteobacterial hydrocarbon degraders or sequences retrieved from hydrocarbon-polluted sediments (Genovese et al. 2014). Therefore, it is probable that

these bacteria were responsible for the low level of biodegradation observed in anoxic sediments. No deltaproteobacterial sequences were observed in oxic sediments, as expected. In the oxic sediments, the authors observed temporal dynamics in the abundance of sequences affiliated to OHCB (*Thalassolituus*, *Alcanivorax*, *Cycloclasticus*) and *Marinobacter*, either with initial decreases immediately after polluting the sediments and/or with transient increases after 1 month of incubation. At the end of the experiment, these bacteria were no longer present in the sediments, because most of the fuel oil was already degraded, presumably by these microorganisms (Genovese et al. 2014). The results of this experiment demonstrate the feasibility of in situ aeration of sediments for bioremediation of harbor sediments. Further experiments have been done in the laboratory to test the effect of aeration together with addition of a slow-release fertilizer and an oil sorbent, as well as the effect of temperature (Cappello et al. 2015).

Finally, a completely different strategy using a bioelectrochemical method has been published recently. This approach consisted in connecting the contaminated anoxic zone of sediments with the overlying oxic water through an electrode. The anodic end of the electrode (at the anoxic sediments) accepts electrons from the oxidation of pollutants or reduced compounds, and these electrons flow to the cathode in the oxic water, eventually reducing oxygen to generate water. The idea is to provide a constant supply of electron acceptor to enhance biodegradation by autochthonous sediment bacteria. This approach has been tested in laboratory microcosms with sediments from Messina harbor (Viggi et al. 2015). A significant reduction in the total hydrocarbon content was observed in microcosms containing graphite electrodes (called "oil-spill snorkels" by the authors). *Delta-*, *Gamma-*, and *Alphaproteobacteria* were observed in biofilms attached to the surface of graphite electrodes (Viggi et al. 2015). A further development of this passive method has been published recently (Bellagamba et al. 2017). In this case, low-voltage currents (2 V) were applied to sediments to cause the electrolysis of water molecules and the generation of oxygen in the sediments. The system was tested again in laboratory microcosms with Messina harbor sediments. The authors demonstrated electrolysis and the generation of oxygen at the anode inserted in the sediment. They also showed that the change in redox potential extended several centimeters (>5 cm) from the anode, indicating that there was a radius of influence around the electrode. The total concentration of hydrocarbons was lower, and degradation rates higher (threefold after 40 days), in microcosms where electric current was applied. In comparison with the controls, *Gammaproteobacteria* (quantified by CARD-FISH) were more abundant in sediments receiving continuous electrical current (Bellagamba et al. 2017). Since many aerobic hydrocarbon degraders belong to *Gammaproteobacteria*, the authors speculated that the electric treatment could stimulated autochthonous degrading bacteria. However, this needs to be confirmed in further experiments and sequencing-based approaches. This type of experiments should help in the design of cost-effective and nontoxic bioremediation procedures for polluted harbor sediments, either enhancing anaerobic or aerobic degradation.

5 Research Needs

The number of studies on microbial communities in harbor environments published in the last decade is remarkable. We have now a better understanding on the taxonomic composition of microbial communities in harbor water and sediments. However, the level of taxonomic resolution in some studies (order, family), particularly in sediment samples, is too low for a good understanding of the complexity of these communities. Besides, there is hardly any data on the composition and function of archaea in harbor sediments. On the other hand, we should take advantage of the reduced costs of some *omics* techniques (i.e., metagenomics) and also increase the number of studies exploring the microbial communities in harbors at the functional level (metatranscriptomics, metaproteomics, and metabolomics). However, we should take into account that generating more sequence data does not necessarily mean better knowledge. More effort should be put into the comparison of the different sequence datasets from harbor environments and those from other chronically polluted environments. This comparison might provide information on the presence of particular populations, ecotypes, or functions (i.e., catabolic pathways) that could be linked to particular environmental factors in harbors. This information could also help to differentiate harbor communities from those in unpolluted environments or those suffering from accidental pollution. This approach might be a way to identify microbial indicators or biomarkers for harbor environments and/or to identify relevant functions from the point of view of bioremediation.

In relation to the degradation of hydrocarbons by harbor communities, we need to solve several key questions: (1) what are the in situ hydrocarbon-degradation rates in water and sediments, (2) which microorganisms are degrading hydrocarbons or catabolic intermediates in situ, (3) what is the coupling between water and sediment communities, and (4) what are the effects of other pollutants (i.e., heavy metals) in hydrocarbon degradation. The use of stable-isotope methods could help to shed light on the first two questions. In addition, the composition of degrading networks, and the catabolic processes involved, might be approached using a combination of *omics* techniques and methods derived from network analysis, as has been shown in this chapter. The effect of heavy metals on hydrocarbon degradation network could be easily addressed, at least at the level of microcosm experimentation. This would be a good starting point to understand the combined effect of hydrocarbons and heavy metals on microbial communities of harbors. Finally, we do not have any data on the third question, i.e., the possible relationships between water and sediment communities. Water retention times in harbors are higher than in open environments, and water depth is not particularly high in most harbors. Therefore, we can expect strong coupling of water and sediment compartments. Although from some harbors (i.e., Messina) we have information from both compartments, the studies were done at different time points. There is no single study known to the author that analyzes water and sediment communities in a harbor at the same sampling time. We could start by analyzing taxonomic and functional diversity in water and sediment samples

taken at the same time points. With good support from measures of relevant environmental parameters, we might be able to better understand the reasons that rule the diversity and dynamics of harbor communities if we consider these environments from a global perspective.

References

Acosta-González A, Marqués S (2016) Bacterial diversity in oil-polluted marine coastal sediments. Curr Opin Biotechnol 38:24–32.

Aguiló-Ferretjans MM, Bosch R, Martín-Cardona C, Lalucat J, Nogales B (2008) Phylogenetic analysis of the composition of bacterial communities in human-exploited coastal environments from Mallorca Island (Spain). Syst Appl Microbiol 31:231–240.

Barbato M, Mapelli F, Magagnini M, Chouaia B, Armeni M, Marasco R, Crotti E, Daffonchio D, Borin S (2016) Hydrocarbon pollutants shape bacterial community assembly of harbor sediments. Mar Pollut Bull 104:211–220.

Bargiela R, Mapelli F, Rojo D, Chouaia B, Tornés J, Borin S, Richter M, Del Pozo MV, Cappello S, Gertler C, Genovese M, Denaro R, Martínez-Martínez M, Fodelianakis S, Amer RA, Bigazzi D, Han X, Chen J, Chernikova TN, Golyshina OV, Mahjoubi M, Jaouanil A, Benzha F, Magagnini M, Hussein E, Al-Horani F, Cherif A, Blaghen M, Abdel-Fattah YR, Kalogerakis N, Barbas C, Malkawi HI, Golyshin PN, Yakimov MM, Daffonchio D, Ferrer M (2015a) Bacterial population and biodegradation potential in chronically crude oil-contaminated marine sediments are strongly linked to temperature. Sci Rep 5:11651.

Bargiela R, Herbst F-A, Martínez-Martínez M, Seifert J, Rojo D, Cappello S, Genovese M, Crisafi F, Denaro R, Chernikova TN, Barbas C, von Bergen M, Yakimov MM, Ferrer M, Golyshin PN (2015b) Metaproteomics and metabolomics analyses of chronically petroleum-polluted sites reveal the importance of general anaerobic processes uncoupled with degradation. Proteomics 15:3508–3520.

Bargiela R, Gertler C, Magagnini M, Mapelli F, Chen J, Daffonchio D, Golyshin PN, Ferrer M (2015c) Degradation network reconstruction in uric acid and ammonium amendments in oil-degrading marine microcosms guided by metagenome data. Front Microbiol 6:article 1270.

Bellagamba M, Viggi CC, Ademollo N, Rossetti S, Aulenta F (2017) Electrolysis-driven bioremediation of crude oil-contaminated marine sediments. New Biotechnol 38:84–90.

Birch GF (2017) Assessment of human-induced change and biological risk posed by contaminants in estuarine/harbour sediments: Sydney Harbour/estuary (Australia). Mar Pollut Bull 116:234–248.

Cappato A, Canevello S, Baggiani B (2011) Cruises and recreational boating in the Mediterranean. Plan Bleu, Valbone

Cappello S, Caruso G, Zampino D, Monticelli LS, Maimone G, Denaro R, Tripodo B, Troussellier M, Yakimov M, Giuliano L (2007a) Microbial community dynamics during assays of harbour oil spill bioremediation: a microscale simulation study. J Appl Microbiol 102:184–194

Cappello S, Denaro R, Genovese M, Giuliano L, Yakimov MM (2007b) Predominant growth of *Alcanivorax* during experiments on "oil spill bioremediation" in mesocosms. Microbiol Res 162:185–190

Cappello S, Genovese M, Della Torre C, Crisari A, Hassanshahian M, Santisi S, Calogero R, Yakimov MM (2012) Effect of bioemulsificant exopolysaccharide (EPS_{2003}) on microbial community dynamics during assays of oil spill bioremediation: a microcosm study. Mar Pollut Bull 64:2820–2828

Cappello S, Calogero R, Santisi S, Genovese M, Denaro R, Genovese L, Giuliano L, Mancini G, Yakimov MM (2015) Bioremediation of oil polluted marine sediments: a bio-engineering treatment. Int Microbiol 18:127–134

Caruso G, La Ferla R, Azzaro M, Zoppini A, Marino G, Petochi T, Corinaldesi C, Leonardi M, Zaccone R, Umani SF, Caroppo C, Monticelli L, Azzaro F, Decembrini F, Maimone G, Cavallo

RA, Stabili L, Todorova NH, Karamfilov VK, Rastelli E, Cappello S, Acquaviva MI, Narracci M, De Angelis R, Del Negro P, Latini M, Danovaro R (2016) Microbial assemblages for environmental quality assessment: knowledge, gaps and usefulness in the European Marine Strategy Framework Directive. Crit Rev Microbiol 42:883–904

Chan JTK, Leung HM, Yue PYK, Au CK, Wong YK, Cheung KC, Li WC, Yung KKL (2017) Combined effects of land reclamation, channel dredging upon the bioavailable concentration of polycyclic aromatic hydrocarbons (PAHs) in Victoria Harbour sediment, Hong Kong. Mar Pollut Bull 114:587–591

Chiellini C, Iannelli R, Verni F, Petroni G (2013) Bacterial communities in polluted seabed sediments: a molecular biology assay in Leghorn harbor. Sci World J 2013:article ID 165706

Coates JD, Woodward J, Allen J, Philp P, Lovley DR (1997) Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. Appl Environ Microbiol 63:3589–3593

Cutroneo L, Carbone C, Consani S, Vagge G, Canepa G, Capello M (2017) Environmental complexity of a port: evidence from circulation of the water masses, and composition and contamination of bottom sediments. Mar Pollut Bull 119:184–194

Dell'Anno A, Beolchini F, Gabellini M, Rocchetti L, Pusceddu A, Danovaro R (2009) Bioremediation of petroleum hydrocarbons in anoxic marine sediments: consequences on the speciation of heavy metals. Mar Pollut Bull 58:1808–1814

Dell'Anno A, Beolchini F, Rocchetti L, Luna GM, Danovaro R (2012) High bacterial biodiversity increases degradation performance of hydrocarbons during bioremediation of contaminated harbor sediments. Environ Pollut 167:85–92

Denaro R, D'Auria G, Di Marco G, Genovese M, Troussellier M, Yakimov MM, Giuliano L (2005) Assessing terminal restriction fragment length polymorphism suitability for the description of bacterial community structure and dynamics in hydrocarbon-polluted marine environments. Environ Microbiol 7:78–87

Duran R, Bielen A, Paradžik T, Gassie C, Pustijanac E, Cagnon C, Hamer B, Vujaklija D (2015) Exploring *Actinobacteria* assemblages in coastal marine sediments under contrasted human influences in the West Istria Sea, Croatia. Environ Sci Pollut Res 22:15215–15229

ECORYS (2013) Study in support of policy measures for maritime and coastal tourism at EU level. ECORYS Nederland BV. Rotterdam

Genovese M, Crisafi F, Denaro R, Cappello S, Russo D, Calogero R, Santisi S, Catalfamo M, Modica A, Smedile F, Genovese L, Golyshin PN, Giuliano L, Yakimov MM (2014) Effective bioremediation strategy for rapid in situ cleanup of anoxic marine sediments in mesocosms oil spill simulation. Front Microbiol 5:article 162

Gertler C, Bargiela R, Mapelli F, Han X, Chen J, Hai T, Amer RA, Mahjoubi M, Malkawi H, Magagnini M, Cherif A, Abdel-Fattah YR, Kalogerakis N, Daffonchio D, Ferrer M, Golyshin P (2015) Conversion of uric acid into ammonium in oil-degrading marine microbial communities: a possible role of halomonads. Microb Ecol 70:724–740

Gomes NCM, Manco SC, Pires ACC, Gonçalves SF, Calado R, Cleary DFR, Loureiro S (2013) Richness and composition of sediment bacterial assemblages in an Atlantic port environment. Sci Total Environ 452–453:172–180

Gómez AG, Bárcena JF, Juanes JA, Ondiviela B, Sámano ML (2014) Transport time scales as physical descriptors to characterize heavily modified water bodies near ports in coastal zones. J Environ Manag 136:76–84

Gómez AG, Ondiviela B, Puente A, Juanes JA (2015) Environmental risk assessment of water quality in harbor areas: a new methodology applied to European ports. J Environ Manag 155:77–88

Grifoll M, Jordà G, Espino M (2014) Surface water renewal and mixing mechanisms in a semi-enclosed microtidal domain. The Barcelona harbour case. J Sea Res 90:54–63

Gutierrez T (2017) Marine, aerobic hydrocarbon-degrading *Gammaproteobacteria*: overview. In: McGenity TJ (ed) Taxonomy, genomics and ecophysiology of hydrocarbon-degrading microbes: handbook of hydrocarbon and lipid microbiology, 2nd edn. Springer, Cham. https://doi.org/10.1007/978-3-319-60053-6_22-1

Hayes LA, Lovley DR (2002) Specific 16S rDNA sequences associated with naphthalene degradation under sulfate-reducing conditions in harbor sediments. Microb Ecol 43:134–145

Hayes LA, Nevin KP, Lovley DR (1999) Role of prior exposure on anaerobic degradation of naphthalene and phenanthrene in marine harbor sediments. Org Geochem 30:937–945

International Maritime Organisation (2003) MARPOL – how to do it. Manual on the practical implications of ratifying, implementing and enforcing MARPOL 73/78, 2002 edition. International Maritime Organization, London

International Maritime Organisation (2014) Consolidated guidance for port reception facility providers and users. MEPC.1/Circ.834. International Maritime Organization. London

Jeanbille M, Gury J, Duran R, Tronczynski J, Agogué H, Saïd OB, Ghiglione J-F, Auguet J-C (2016) Response of core microbial consortia to chronic hydrocarbon contaminations in coastal sediment habitats. Front Microbiol 7:article 1637

Johnston EL, Roberts DA (2009) Contaminants reduce the richness and eveness of marine communities: a review and meta-analysis. Environ Pollut 157:1745–1752

Kalogerakis N, Arff J, Banat IM, Broch OJ, Daffonchio D, Edvardsen T, Eguiraun H, Giuliano L, Handå A, López-de-Ipiña K, Marigomez I, Martinez I, Øie G, Rojo F, Skjermo J, Zanaroli G, Fava F (2015) The role of environmental biotechnology in exploring, exploiting, monitoring, preserving, protecting and decontaminating the marine environment. New Biotechnol 32:157–167

Kan J, Wang K, Chen F (2006) Temporal variation and detection limit of an estuarine bacterioplankton community analyzed by denaturing gradient gel electrophoresis (DGGE). Aquat Microb Ecol 42:7–18

King AJ, Readman JW, Zhou JL (2004) Dynamic behaviour of polycyclic aromatic hydrocarbons in Brighton Marina, UK. Mar Pollut Bull 48:229–239

Kisand V, Valente A, Lahm A, Tanet G, Lettieri T (2012) Phylogenetic and functional metagenomic profiling for assessing microbial biodiversity in environmental monitoring. PLoS One 7:e43630

Misson B, Garnier C, Lauga B, Dang DH, Ghiglione J-F, Mullot J-U, Duran R, Pringault O (2016) Chemical multi-contamination drives benthic prokaryotic diversity in the anthropized Toulon Bay. Sci Total Environ 556:319–329

Neira C, Cossaboon J, Mendoza G, Hoh E, Levin LA (2017) Occurrence and distribution of polycyclic aromatic hydrocarbons in surface sediments of San Diego Bay marinas. Mar Pollut Bull 114:466–479

Ng C, Le T-H, Goh SG, Liang L, Kim Y, Rose JB, Yew-Hoong KG (2015) A comparison of microbial water quality and diversity for ballast and tropical harbor waters. PLoS One 10: e0143123

Nogales B, Aguiló-Ferretjans MM, Martín-Cardona C, Lalucat J, Bosch R (2007) Bacterial diversity, composition and dynamics in and around recreational coastal areas. Environ Microbiol 9:1913–1929

Pérez S, Dachs J, Barceló D (2003) Sea breeze modulated volatilization of polycyclic aromatic hydrocarbons from the Masnou Harbor (NW Mediterranean Sea). Environ Sci Technol 37:3794–3802

Pinheiro PPO, Massone CG, Carreira RS (2017) Distribution, sources and toxicity potential of hydrocarbons in harbor sediments: a regional assessment in SE Brazil. Mar Pollut Bull 120:6–17

Rocchetti L, Beolchini F, Hallberg KB, Johnson DB, Dell'Anno A (2012) Effects of prokaryotic diversity changes on hydrocarbon degradation rates and metal partitioning during bioremediation of contaminated anoxic marine sediments. Mar Pollut Bull 64:1688–1698

Rothermich MM, Hayes LA, Lovey DR (2002) Anaerobic, sulfate-dependent degradation of polycyclic aromatic hydrocarbons in petroleum-contaminated harbor sediment. Environ Sci Technol 36:4811–4817

Sauret C, Tedetti M, Guigue C, Dumas C, Lami R, Pujo-Pay M, Conan P, Goutx M, Ghiglione J-F (2016) Influence of PHAs among other coastal environmental variables on total and PAH-degrading bacterial communities. Environ Sci Pollut Res 23:4242–4256

Schauer M, Massana R, Pedrós-Alió C (2000) Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fingerprinting. FEMS Microb Ecol 33:51–59

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl G (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci U S A 103:12115–12120

Tavares TCL, Normando LRO, de Vasconcelos ATR, Gerber AL, Agnez-Lima LF, Melo VMM (2016) Metagenomic analysis of sediments under seaports influence in the Equatorial Atlantic Ocean. Sci Total Environ 557–558:888–900

UNCTAD, United Nations Conference of Trade and Development (2016) Review of maritime transport. United Nations, Geneva

Viggi CC, Presta E, Bellagamba M, Kaciulis S, Balijepalli SK, Zanaroli G, Papini MP, Rossetti S, Aulenta F (2015) The "oil spill snorkel": an innovative bioelectrochemical approach to accelerate hydrocarbons biodegradation in marine sediments. Front Microbiol 6:article 881

Wu X, Monchy S, Taghavi S, Zhu W, Ramos J, van der Lelie D (2011) Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*. FEMS Microbiol Rev 35:299–323

Yakimov MM, Denaro R, Genovese M, Cappello S, D'Auria G, Chernikova TN, Timmis KN, Golyshin PN, Giuliano L (2005) Natural microbial diversity in superficial sediments of Milazzo Harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons. Environ Microbiol 7:1426–1441

Zhang R, Liu B, Lau SCK, Ki J-S, Qian P-Y (2007) Particle-attached and free-living bacterial communities in a contrasting marine environment: Victoria Harbor, Hong Kong. FEMS Microbiol Ecol 61:496–508

Zhang W, Ki J-S, Qian P-Y (2008) Microbial diversity in polluted harbor sediments I: bacterial community assessment based on four clone libraries of 16S rDNA. Estuar Coast Shelf Sci 76:668–681

Zhang R, Lau SCK, Ki J-S, Thiyagarajan V, Qian P-Y (2009) Response of bacterioplankton community structures to hydrological conditions and anthropogenic pollution in contrasting subtropical environments. FEMS Microbiol Ecol 69:449–460

Hydrocarbon-Degrading Microbial Communities in Natural Oil Seeps

6

Andreas Teske

Contents

Abstract

Hydrocarbons in natural oil seeps provide carbon and energy sources for extensive and diverse microbial communities. This chapter provides an introduction to sulfate-reducing, methanogenic, and methane- or alkane-oxidizing anaerobic microbial populations from hydrocarbon-rich marine habitats. Persistent enrichment and cultivation efforts and pure culture studies have greatly increased the recognized diversity of cultured, hydrocarbon-oxidizing microorganisms and the knowledge of their substrate spectra, habitat preferences, and ecophysiological function in their natural environments. This chapter highlights model ecosystems where diverse hydrocarbon-oxidizing microbial communities are sustained by fossil hydrocarbons; characteristic examples are the hydrocarbon seeps in the

A. Teske
Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
e-mail: teske@email.unc.edu

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_3

Gulf of Mexico, where deeply sourced hydrocarbons of Jurassic origin are rising through extensive sediment layers that are fractured by salt tectonics, and the hydrothermal Guaymas Basin in the Gulf of California, where buried organic matter of photosynthetic origin, which is undergoing thermal maturation, is transformed into young petroleum within the upper sediment layers. These examples provide surface expressions of subsurface hydrocarbon reservoirs and their microbiota. The microbial communities in hydrocarbon seeps and reservoirs reflect the spectrum of carbon substrates and electron donors and the wide range of physical conditions that characterize these habitats.

1 Introduction

Microbial life thrives in marine sediments and in marine hydrocarbon deposits and seeps (Jørgensen and Boetius 2007). As hydrocarbons migrate upward from their deep subsurface source to the sediment-water interface, they fuel benthic hydrocarbon-degrading microbial communities containing physiologically and phylogenetically diverse anaerobes, such as sulfate reducers, methanogens, methane oxidizers, and fermenters. Hydrocarbon-rich habitats provide a hunting ground for detecting and characterizing novel physiological and phylogenetic types of hydrocarbon-degrading bacteria and archaea, as demonstrated already 20 years ago when six novel bacterial phyla were discovered in a 16S rRNA gene sequencing survey of a terrestrial aquifer contaminated with jet fuel-derived hydrocarbons and chlorinated solvents (Dojka et al. 1998). Two contrasting marine hydrocarbon-based ecosystems provide particularly instructive model cases: the hydrothermal sediments of Guaymas Basin in the Gulf of California, where high temperatures and pressure transform photosynthetic biomass embedded in the upper sediment layers into young hydrothermal petroleum, and the Gulf of Mexico, where deeply sourced hydrocarbons of Jurassic origin are rising through massive continental slope sediments that are fractured by salt tectonics. These locations will provide the starting point for exploring the environmental diversity of anaerobic alkane- and aromatics-oxidizing bacteria and archaea that have been obtained from these marine model systems and other hydrocarbon-rich habitats. To place practical limits on this far-ranging subject that could easily be treated more comprehensively and at greater length, this chapter focuses primarily on sulfate-reducing, methane-cycling, short-chain alkane- and aromatics-oxidizing microbial communities.

2 Hot Hydrocarbon Seepage in Guaymas Basin

The Guaymas Basin in the Gulf of California is a young marginal rift basin characterized by active seafloor spreading and rapid deposition of photosynthetic biomass and phytoplankton, in particular diatoms, from highly productive overlying

waters, supplemented locally by terrigenous sedimentation from the Sonora Margin (Calvert 1966; Schrader 1982). The northern and southern axial troughs of Guaymas Basin are bounded by extensive axial-parallel fault lines on both sides (Lonsdale and Becker 1985; Fisher and Becker 1991). Organic-rich sediments of several hundred meters thickness overlie the spreading centers of Guaymas Basin and alternate with shallow intrusions of doleritic sills into the unconsolidated sediments (Einsele et al. 1980). Whereas spreading centers at mid-ocean ridges lacking sediment cover favor focused magma emplacement and exposure to cold seawater directly at the spreading center, the sediments in Guaymas Basin act as a thermal blanket that allows broader off-axis magmatism which extends into axial-parallel faults (Berndt et al. 2016) and the sedimented ridge flanks (Lizarralde et al. 2011). Emplacement of hot sills indurates and hydrothermally alters the sediment layers above and below the sill; buried sills continue to shape hydrothermal circulation patterns and reaction pathways (Saunders et al. 1982; Gieskes et al. 1982; Kastner 1982).

This juxtaposition of active seafloor spreading, sill emplacement, and rapid sedimentation creates a dynamic environment where physical, chemical, and microbiological processes regulate the cycling of sedimentary carbon, in particular the synthesis, mobilization, and microbial utilization of fossil hydrocarbons. Buried organic matter in the Guaymas sediments is heated at ca. 300 °C under high pressure and transformed quickly into hydrocarbons, a hydrothermal process that has also been studied in the laboratory (Seewald et al. 1994). Guaymas Basin petroleum is young enough to be ^{14}C-dated; it has an average radiocarbon age of approximately 5000 years (Peter et al. 1991). Hydrothermal pyrolysis transforms and mobilizes a major proportion of subsurface carbon sources: The organic carbon content of approx. 3–4 wt% in surficial Guaymas Basin sediments (De la Lanza-Espino and Soto 1999) is reduced to 1–2% in indurated and heated subsurface sediments below sills (Rullkötter et al. 1982; Simoneit and Bode 1982). Mobilization and expulsion of sedimentary organic matter by hydrothermal heating are also evident in surficial sediments of Guaymas Basin (Lin et al. 2017). The resulting, hydrothermally altered fluids that are reaching the sediment surface are enriched in thermally generated hydrocarbons that are derived from buried organic matter; these include aromatic compounds, alkanes and methane (Galimov and Simoneit 1982; Kawka and Simoneit 1987; Whelan et al. 1988; Welhan et al. 1988; Bazylinski et al. 1988; Didyk and Simoneit 1989), and low-molecular-weight organic acids (Martens 1990). Transport of hydrocarbon-rich fluids through the upper sediment column and to the sediment-water interface directly links geological, physical, and biogeochemical processes by providing fossil carbon substrates to a highly active, benthic microbial ecosystem (Pearson et al. 2005). Highly active and complex benthic microbial communities occur within a narrow, near-surface sediment horizon (Götz and Jannasch 1993; Guezennec et al. 1996; Teske et al. 2002; Teske et al. 2003; Edgcomb et al. 2002; Biddle et al. 2012; McKay et al. 2012, 2016). Upward substrate and temperature fluxes in Guaymas Basin create a spatially compressed zone of anaerobic microbial processes and communities just below and on the sediment surface, including methanogenesis (Welhan et al. 1988), anaerobic methane oxidation

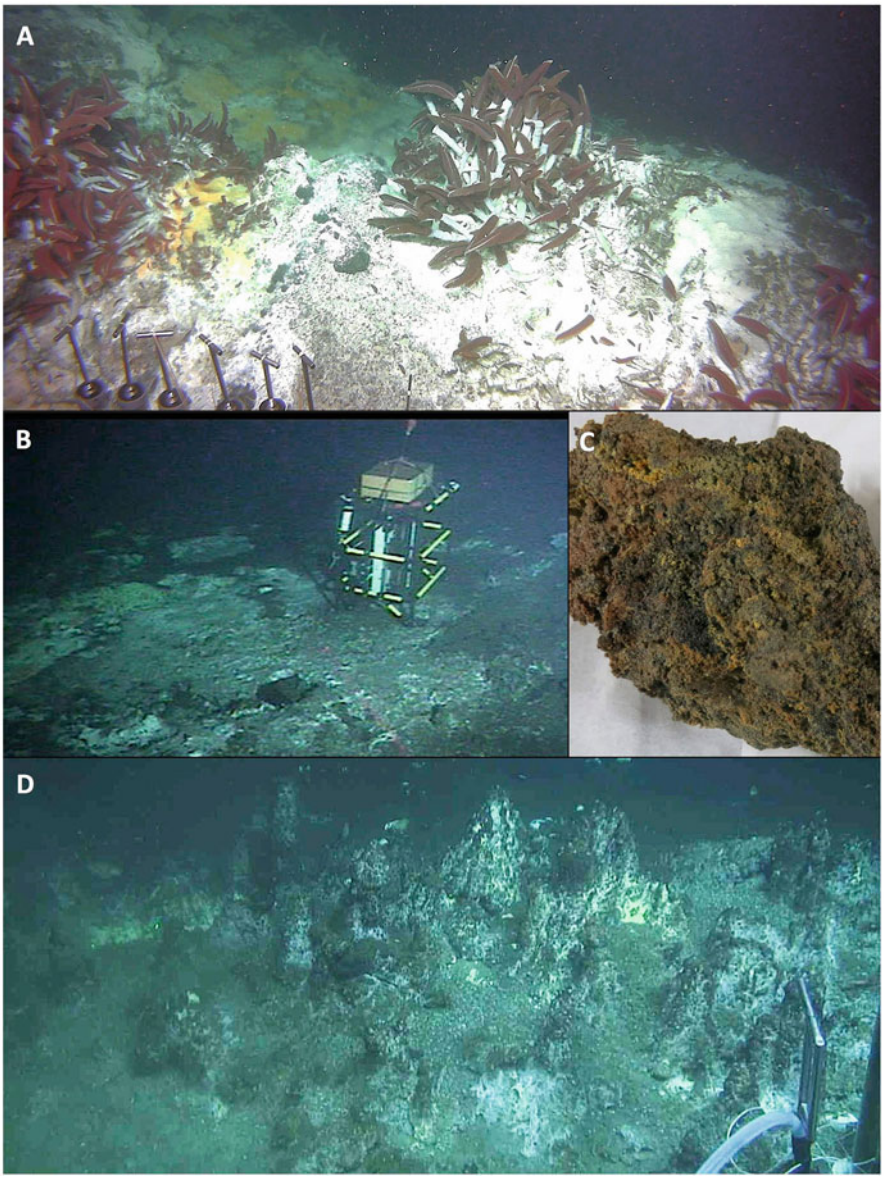

Fig. 1 Hydrocarbon seepage in Guaymas Basin. (**a**) Classical hydrothermal seafloor landscape of Guaymas Basin with orange and white sulfur-oxidizing *Beggiatoaceae* mats, sulfur precipitates, and *Riftia* tube worms at the southern spreading center. *Alvin* Dive 4868, December 19, 2016. (**b**). Hydrocarbon-rich, hot sediment at the Megamat site (Teske et al. 2016), with in situ microelectrode profiler in the background. These sediments contained ample liquid hydrocarbons and up to 1–2 mM pentane and hexane [M. Kellermann, unpubl. results]. *Alvin* dive 4486. December 9, 2008. (**c**) Petroleum-stained seafloor mineral accretion. The sample is approx. 10 cm in diameter and

(Kallmeyer and Boetius 2004), sulfate reduction (Elsgaard et al. 1994; Jørgensen et al. 1990, 1992; Kallmeyer et al. 2003; Weber and Jørgensen 2002), and sulfide oxidation (Jannasch et al. 1989; Nelson et al. 1989; Gundersen et al. 1992). These hot spots of microbial activity reflect the subsurface flow patterns and localized supply of organic substrates and inorganic electron donors in Guaymas Basin sediments; visual inspection and subsequent geochemical and microbiological analysis of conspicuous mat-covered sediments show a complex landscape of sulfide-oxidizing microbial mats on hydrothermal sediments with sulfate-reducing and methane-cycling communities (McKay et al. 2012, 2016; Teske et al. 2016). Interestingly, the hydrothermal sediments and seafloor mineral concretions that are rich in alkanes [beyond methane] and liquid petroleum often lack the conspicuous overgrowth of white, yellow, and orange microbial mats and instead show muted, light-gray or off-white surface dustings with a powdery-looking texture and the occasional lime-yellow patches of elemental sulfur (Fig. 1). In such cases, not microbial mat growth, but sulfur accumulation at the sediment-water interface may dominate the surface color and appearance (Teske et al. 2016).

Briefly summarized for introductory purposes, these sediments are the source of numerous sulfate-reducing hydrocarbon oxidizers, often thermophilic or thermotolerant isolates, such as the decane-oxidizing bacterium *Desulfothermus naphthae* strain TD3 (Rüter et al. 1994), a propane-oxidizing bacterial enrichment dominated by *Desulfotomaculum* strain Propane60-GuB (Kniemeyer et al. 2007), thermophilic methane-oxidizing archaeal enrichments dominated by members of the anaerobic methane-oxidizing (ANME-1) archaeal lineage (Holler et al. 2011, Wegener et al. 2015), by the thermophilic butane-oxidizing archaeon *Candidatus* Syntrophoarcheum sp. (Laso-Pérez et al. 2016), and by the thermophilic, hydrogenotrophic sulfate-reducing bacterium *Candidatus* Desulfofervidus auxilii that grows in syntrophic association with methane- and butane-oxidizing archaea (Krukenberg et al. 2016). Mesophilic sulfate-reducing isolates from Guaymas Basin include the *n*-butane and propane oxidizer BuS5 within the family *Desulfobacteraceae* (Kniemeyer et al. 2007), and strain EbS7 within the *Desulfatiglans* lineage, capable of complete oxidation of ethylbenzene (Kniemeyer et al. 2003). Stable isotope labeling experiments with butane and dodecane have identified phylotypes forming sister lineages to the genera *Desulfosarcina*, *Desulfococcus*, and *Desulfonema* within the *Desulfobacteraceae* (Kleindienst et al. 2014). Sequence-based screening of aromatics [benzene] enrichments from Guaymas Basin sediments has recovered further sequences of the *Desulfobacteraceae* and the *Desulfatiglans* lineage (Phelps et al. 1998) (Fig. 1).

Fig. 1 (continued) comes from the petroleum-soaked miniature spires (**d**), ca. 20 cm high, found during the eastern approach toward "Rebecca's Roost," a massive hydrothermal edifice (Teske et al. 2016). *Alvin* dive 4872, December 24, 2016. Thick orange and white *Beggiatoaceae* are frequently found on hot sediments and rocks with sulfide and methane-rich hydrothermal fluids, but they are poorly developed or missing on these hydrocarbon-rich sediments and mineral accretions

3 Cold Hydrocarbon Seepage in the Gulf of Mexico

The sediments of the continental slope of the northern Gulf of Mexico contain large reservoirs of petroleum and gas that sustain an arc of seafloor hydrocarbon seeps from offshore Mississippi to Texas. These seafloor environments are characterized by petroleum leakage and channelized gas flux and ebullition from the seafloor (Kennicutt et al. 1988), methane-soaked seep sediments dominated by anaerobic methane oxidation (Lapham et al. 2008), seafloor methane hydrate formation (Brooks et al. 1984; Sassen et al. 1999) often in association with oil seepage (Brooks et al. 1986), sediment and gas flow from large seafloor mud volcanoes (McDonald et al. 2000), and methane-derived authigenic carbonates (Roberts et al. 2010a). Frequently, hydrocarbon seepage on the continental slope of the northern Gulf of Mexico shows admixtures of subseafloor brine. The Mid-Jurassic Louann evaporite formation, predominantly halite, extends from Texas to the Florida panhandle underneath the northern Gulf slope, coast, and coastal plain (Amos 1987). These salt formations have a lower density than the overlying compacted marine sediments (Lerche and Petersen 1995); since they are incompressible, they gradually move and buckle upward through the surrounding and overlying sediment layers and create structurally complex salt dome crests and mounds in the overlying seafloor (Roberts et al. 1990) that result in a highly conspicuous and characteristic, topsy-turvy seafloor bathymetry (Bryant et al. 1990). Fractures within the overlying sediment then provide a network of pathways for upward migration of subsurface hydrocarbons (Roberts et al. 1999). In consequence of their prolonged contact with subsurface salt formations, rising hydrocarbon-rich seep fluids can be highly saline; they collect in seafloor depressions as quiescent, anoxic hydrocarbon-rich brine lakes with a stable halocline and redoxcline at the brine/seawater interface that favors colonization by chemosynthetic invertebrate communities, for example, extensive mussel banks (MacDonald et al. 1990). Under more active flow conditions, hydrocarbon-rich brines entrain fluidized sediments and create pulsating saline mud volcanoes that frequently overflow their seafloor basin and send a network of briny rivulets running downslope over the surrounding seafloor (McDonald et al. 2000; Joye et al. 2005). Seafloor brine lakes and mud volcanoes can transition into each other; a currently quiescent brine lake can have a dynamic past as a mud volcano (MacDonald and Peccini 2009) and can again erupt as a mud volcano after extended quiescence or in irregular intervals (MacDonald et al. 2000). Obviously, briny seafloor lakes and mud volcanoes provide habitat for halophiles and extreme halophiles and select for halophilic or at least halotolerant variants of hydrocarbon-utilizing or chemosynthetic bacteria and archaea (Lloyd et al. 2006; Joye et al. 2009).

In some cases, benthic hydrocarbon seepage in the Gulf of Mexico is visible even from the sea surface. The Green Canyon 600 area on the upper continental slope of the Gulf of Mexico harbors a seafloor ridge with the most productive natural oil seeps in the northern Gulf of Mexico, also known as Oil Mountain. Here, oil and gas bubbles rising from the seafloor at 1200 m depth produce prolific

oil slicks that extend for tens of kilometers on the sea surface and are visible from space (Garcia-Pineda et al. 2010). The rough seafloor topography with many small mounds and valleys harbors patches of microbial mats, vesicomyid clams, and vestimentiferan tube worms; the seafloor landscape shows hydrocarbon seepage in all its manifestations (Fig. 2). Hydrocarbons are staining surface-breaching gas hydrates so that partially exposed seafloor hydrates stand out in a rich petroleum brown against the lighter-colored sediment cover. Streams of oil-coated gas bubbles emerge through cracks in carbonate pavement, and oil droplets are seeping from the sediment in low-lying pockmarks (Roberts et al. 2010b; Johansen et al. 2017) (Fig. 2).

In contrast to Guaymas Basin, the potential of the Gulf of Mexico for isolating hydrocarbon-oxidizing bacteria and archaea, including halophilic or halotolerant types, is mostly untapped. Molecular surveys have detected highly diverse deltaproteobacterial sulfate-reducing bacteria (Joye et al. 2009; Lloyd et al. 2006, 2010). Sulfate-reducing enrichments with ethane, propane, and *n*-butane have yielded the n-butane-oxidizing enrichment Butane12-Gm2 and the propane-oxidizing enrichment Prop12-GMe, both related to *Desulfosarcina*/*Desulfococcus* (Kniemeyer et al. 2007; Jaekel et al. 2013). Compared to the closely related butane-oxidizing strain BuS5 from hydrothermal Guaymas Basin, these propane- and butane-oxidizing enrichments from Gulf of Mexico cold seeps had a lower optimum temperature for sulfate reduction, 15–20 °C instead of 25–30 °C (Jaekel et al. 2013). Renewed cultivation efforts after the Deepwater Horizon oil spill have focused mostly on aerobic hydrocarbon oxidizers from the water column and weathered surface oil, such as the alphaproteobacterium *Tritonibacter horizontis*, capable of degrading substituted aromatics (Giebel et al. 2016; Klotz et al. 2018), or the obligate polyaromatics oxidizer *Cycloclasticus* and gammaproteobacterial marine heterotrophs with alkane-degrading capabilities (Gutierrez et al. 2013). *Cycloclasticus* populations have also been detected as endosymbionts in mussels and sponges from asphalt-rich Gulf of Mexico hydrocarbon seeps, with the additional twist that these symbiotic communities subsist on short-chain alkanes (Rubin-Blum et al. 2017). Sedimentation of oil-derived marine snow on the seafloor in the Gulf of Mexico in the fall of 2010 has enriched for sulfate- and metal-reducing bacteria of the *Desulfobacteraceae*, *Desulfobulbaceae*, and *Geobacteraceae* (Kimes et al. 2013; Yang et al. 2016). It is intriguing to speculate that these bacteria could have been enriched from seed populations in natural hydrocarbon seeps and have catalyzed benthic hydrocarbon degradation and bioremediation as a natural microbial ecosystem response.

4 Deep Subsurface Habitats

The hot and cold hydrocarbon seeps of Guaymas Basin and the Gulf of Mexico can be viewed as surface expressions of subsurface hydrocarbon reservoirs. Fermentative bacteria occurring in petroleum reservoirs include numerous mesophilic,

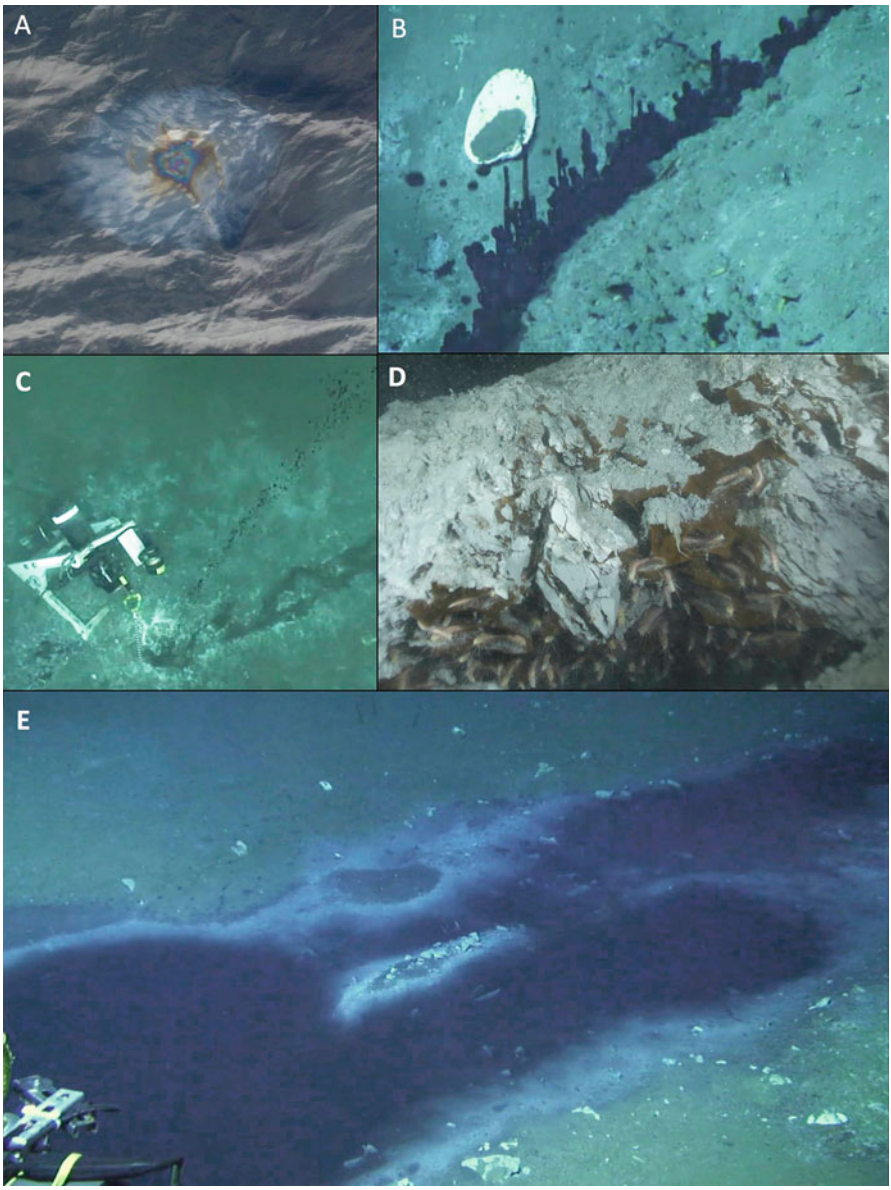

Fig. 2 Hydrocarbon seepage in the Gulf of Mexico. All photos are from Lease Block Green Canyon 660, also known as Oil Mountain, the most productive natural oil seep area in the northern Gulf of Mexico. (**a**) Oil droplet spreading in a rainbow-colored oil sheen on the sea surface after having rising from the seafloor at ca. 1200 m depth; on a calm day, these oil sheens are prominently visible on the sea surface. (**b**) Short strings of dark-brown viscous hydrocarbons, aka the "Birthday Candles," emerge from the sediment near the base of a petroleum-stained hydrate mound. Oil droplets detach periodically from the candle tips and rise into the water column. Photo *Alvin* dive 4689, April 3, 2014. (**c**) Automated seafloor camera by I. MacDonald, Florida State University,

thermophilic, and halophilic strains and species (reviewed in Magot et al. 2000 and Grassia et al. 1996). Pure culture isolates from petroleum reservoirs include mesophilic strains (mostly genus *Desulfovibrio*; Tardy-Jacquenod et al. 1996) and thermophiles, especially members of the gram-positive genus *Desulfotomaculum* and the thermophilic species *Thermodesulforhabdus norvegicus* (Beeder et al. 1995), *Thermodesulfobacterium mobile* (Rozanova and Pivovarova 1988), and *Desulfacinum infernum* (Rees et al. 1995). The thermophilic sulfur- and thiosulfate-reducing, fermentative genera *Geotoga*, *Petrotoga*, and *Thermotoga* are consistently isolated from deep oil fields and appear to be specialized for this habitat (Davey et al. 1993; Jeanthon et al. 1995; Ravot et al. 1995). Petroleum reservoirs have potential for cultivating new lineages of bacteria, for example, the manganese- and iron-reducing thermophile *Deferribacter thermophilus* (Greene et al. 1997).

Many thermophilic sulfate and sulfur reducers are not limited to petroleum reservoirs; their carbon substrate spectra or other physiological features are broadly compatible with this habitat but are not highly specific. For example, thermophilic sulfate reducers of the genus *Desulfotomaculum* are commonly isolated from a wide range of subsurface habitats, with and without petroleum hydrocarbon sources (reviewed in Amend and Teske 2005). Hyperthermophilic archaea of the fermentative, sulfur-reducing genus *Thermococcus* and *Pyrococcus* and of the sulfate-reducing genus *Archaeoglobus* occur in high abundance in deep oil reservoirs around the world (Stetter et al. 1993; Beeder et al. 1994; L'Haridon et al. 1995; Orphan et al. 2000) as well as in hydrothermal vents at mid-ocean spreading centers (Kelley et al. 2002).

Organic substrates that sustain thermophilic microbial populations do not have to undergo hydrothermal processing or fossil hydrocarbon formation. Organic-rich marine subsurface sediments consistently support subsurface life (Parkes et al. 2000). Buried organic matter of photosynthetic origin provides the carbon source for ubiquitous microbial life in marine sediments, as demonstrated for cool continental margin subsurface sediments (Biddle et al. 2006). Even under moderate geothermal heating, recalcitrant buried organic matter in marine sediments undergoes thermal maturation toward greater bioavailability (Wellsbury et al. 1997). Interestingly, 16S rRNA sequencing surveys show that *Thermococcus* and *Pyrococcus* spp. can sometimes be found in deep marine subsurface sediments (Inagaki et al. 2006; Roussel et al. 2008), where they could survive on buried

Fig. 2 (continued) recording a stream of oil droplets emerging from sediment at the Mega Plume site. *Alvin* dive 4690, April 4, 2014. (**d**) A petroleum-stained massive hydrate outcrop, ca. 2 m high. The photo shows a close-up view of the outcrop with ice worms in the crevices of the brown petroleum-stained hydrate, contrasting with the light-gray sediment cover. Cracks and fissures in the sediment cover reveal the dynamics of the underlying hydrate. *Alvin* dive 4690, April 4, 2014. (**e**) A brine flow channel near the petroleum-stained hydrates, surrounded by reducing sediments and a gray-whitish halo of sulfur precipitates that contrast with the dark, sulfidic brine. *Alvin* dive 4696, April 4, 2014. Photo (**a**) by A. Teske, photo (**d**) by I. MacDonald; other photos taken by *Alvin* camera system

organic compounds undergoing thermal maturation. These occurrence patterns suggest a marine geothermal habitat network that includes hydrothermal vents, subsurface petroleum reservoirs, and deep subsurface sediments.

5 Hydrocarbon-Oxidizing Sulfate-Reducing Communities: The SEEP-SRB Lineages

When sulfate is available via seawater inmixing, downward diffusion, or through dissolution of subsurface salt formations and buried evaporates, sulfate-reducing bacteria are thriving in petroleum deposits, hydrocarbon seeps, methane hydrates, petroleum-rich hydrothermal sediments, oil storage tanks, and hydrocarbon-contaminated sediments. The highly diverse sulfate-reducing bacterial communities in these habitats share some broadly characteristic features.

Four 16S rRNA gene clusters of sulfate-reducing bacteria, the SEEP-SRB lineages, are consistently found in methane- and alkane-rich seep habitats (Knittel et al. 2003): the SEEP-SRB1 cluster within the *Desulfobacteraceae* which are generally capable of oxidizing a wide range of substrates, including acetate and CO_2, the independently branching and currently uncultured SEEP-SRB2 lineage which often co-occurs with anaerobic methane-oxidizing (ANME) archaea and has been implicated in alkane oxidation due to its consistent occurrence at alkane-rich seeps (Kleindienst et al. 2012), and the SEEP-SRB3 and SEEP-SRB4 groups within the *Desulfobulbaceae* (Fig. 3) where the Seep-SRB3 lineage is related to syntrophs of the methane-oxidizing archaeal lineage ANME-3 (Lösekann et al. 2007). Fluorescence in situ hybridization (FISH) studies of sulfate-reducing bacteria have identified members of the *Desulfobacteraceae* (including but not limited to SEEP-SRB1) as the dominant group of sulfate-reducing bacteria in seep habitats that are rich in short-chain alkanes (Kleindienst et al. 2012); members of the *Desulfobacteraceae* were also abundantly and consistently detected in propane and butane enrichments analyzed via Nano-SIMS and stable-isotope probing with isotopically labeled alkane substrates (Jaekel et al. 2013; Kleindienst et al. 2014). These results are consistent with numerous 16S rRNA and functional gene sequencing surveys that detect predominantly members of the *Desulfobacteraceae* in marine hydrocarbon seep environments (Dhillon et al. 2003; Lanoil et al. 2001; Lloyd et al. 2006; Mills et al. 2003, 2004, 2005; Teske et al. 2002).

Hydrocarbon- and petroleum-degrading sulfate-reducing communities are compositionally overlapping with those that participate in the syntrophic, sulfate-dependent oxidation of methane to CO_2 (Orphan et al. 2001a, b; Orphan et al. 2002; Niemann et al. 2006; Lösekann et al. 2007). In particular members of the SEEP-SRB1 cluster related to the genera *Desulfosarcina* and *Desulfococcus* overlap with syntrophs of the methane-oxidizing archaea lineages ANME-1 and ANME-2. Highly resolved group-specific FISH and sequencing analyses have identified subgroups of SEEP-SRB1, with the ANME syntrophs clustering specifically in subgroup SEEP-SRB1a (Schreiber et al. 2010); this subgroup is the best example for *Desulfobacteraceae* that are highly specialized and adapted to a syntrophic lifestyle

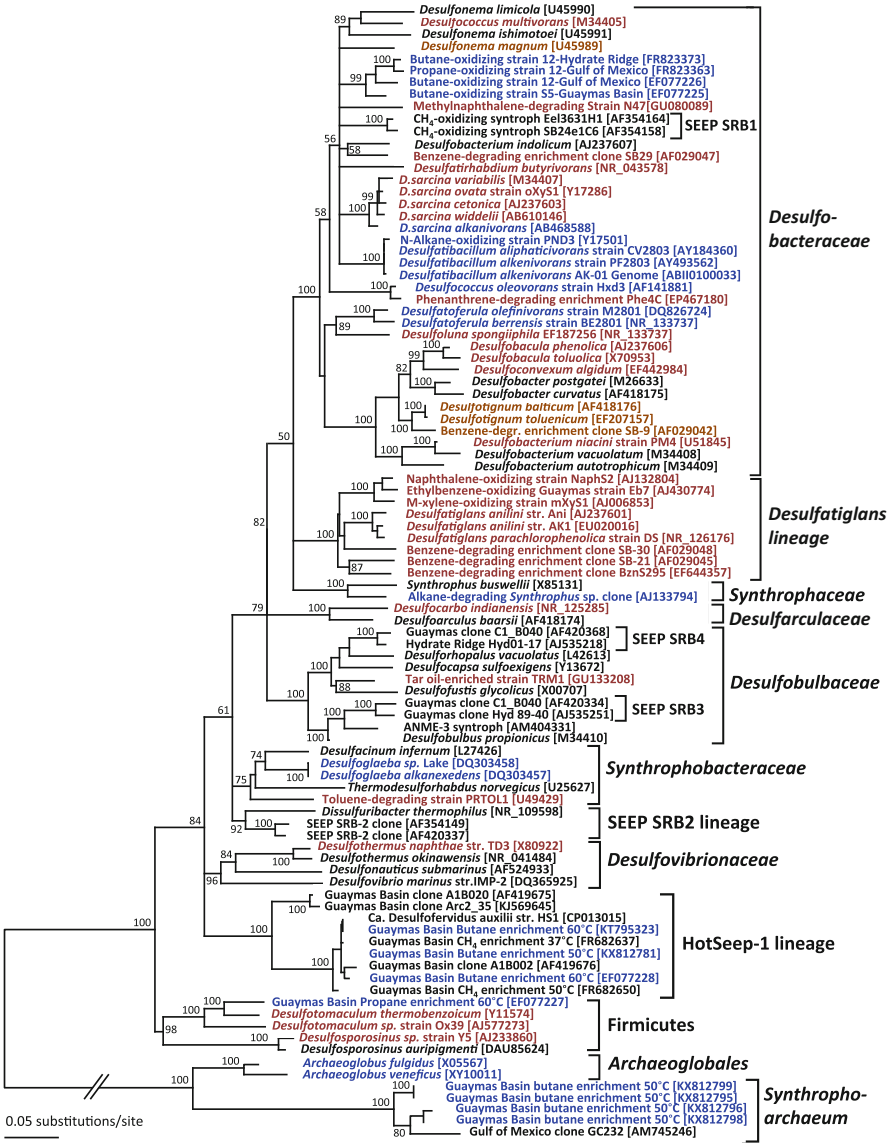

Fig. 3 Phylogenetic tree of hydrocarbon-oxidizing sulfate-reducing bacterial cultures and enrichments, based on near-complete 16S rRNA genes. The tree was rooted with alkane-oxidizing archaea [*Archaeoglobales* and *Syntrophoarchaeum*] as outgroup. Alkane oxidizers are labeled in blue, aromatics oxidizers in dark purple. The taxonomic designations correspond to the taxonomic outline in Bergey's Manual of Systematic Bacteriology, 2nd edition, amended by the Hotseep-1, *Desulfatiglans*, SEEP-SRB1–4, and *Syntrophoarchaeum* lineages. The tree was inferred as a neighbor-joining consensus tree, using distance [minimum evolution] as optimality criterion in PAUP4.0 (HKY85 model, shape parameter 0.5)

in methane oxidation. Sulfate-reducing degraders of other hydrocarbons can be found in other subclusters, outside the SEEP-SRB1 group but still within the *Desulfobacteraceae*, as indicated by FISH studies of alkane-rich marine seep sediments (Kleindienst et al. 2012). In habitats that are rich in complex petroleum-derived substrates, most of the in situ sulfate-reducing activity is coupled to the oxidation of non-methane substrates, for example, low-molecular-weight organic acids (Orcutt et al. 2005; Bowles et al. 2011). The sulfate-reducing community reflects this wide spectrum of substrates and includes numerous types of *Deltaproteobacteria* instead of, or in addition to, sulfate reducers participating in methane oxidation as archaeal syntrophs (Lloyd et al. 2006, 2010).

6 Alkane-Oxidizing Sulfate-Reducing Bacteria

Novel sulfate-reducing bacteria that oxidize petroleum compounds, such as alkanes and aromatic compounds, are specifically adapted to hydrocarbon-rich habitats. The petroleum-rich hydrothermal sediments of Guaymas Basin, of the Gulf of Mexico, and of hydrocarbon-contaminated marine sediments have yielded sulfate-reducing bacteria that specialize in the complete anaerobic oxidation of short-chain alkanes to CO_2. Most of the currently known alkane-oxidizing sulfate-reducing bacteria are related to the cultured genera *Desulfosarcina*, *Desulfococcus*, and *Desulfonema* within the *Desulfobacteraceae* (Fig. 3). Within the genus *Desulfosarcina*, the new alkane-oxidizing species *Desulfosarcina alkanivorans* strain PL12 and the new *p*-xylene-degrading species *Desulfosarcina widdelii* strain PP31 have been isolated and described from oil-polluted marine sediments in Kuwait (Watanabe et al. 2017). An alkane-degrading species of the genus *Desulfococcus*, *D. oleovorans* strain Hxd3, has been isolated on hexadecane (Aeckersberg et al. 1991). The two butane-oxidizing strains Butane 12-Gme, the dominant component of an enrichment culture from the Gulf of Mexico, and pure culture strain BuS5 from Guaymas Basin (Kniemeyer et al. 2007) are closely related to *Desulfosarcina* and *Desulfococcus* spp., but they remain currently without genus and species designation. Several alkane oxidizers with near-identical 16S rRNA sequences fall into the new genus *Desulfatibacillum*: the two species *D. aliphaticivorans* and *alkenivorans* (Cravo-Laureau et al. 2004a, b) and the alkane-oxidizing sulfate-reducing strains Pnd3 (Aeckersberg et al. 1998) and AK-01 (So and Young 1999). These cultured members of the genus *Desulfatibacillum* oxidize alkanes in the C_{12}–C_{23} range and alkenes in the C_8–C_{23} range, with species-specific length preferences. The genus and species *Desulfatiferula olefinivorans* isolated from oil refinery wastewater incompletely oxidizes long-chain (C_{14}–C_{23}) alkenes and fatty acids (Cravo-Laureau et al. 2007). The second species of this genus, *Desulfatiferula berrensis*, was isolated from oil-polluted estuarine sediments and has a similar substrate profile with C_{12}–C_{20} n-alkenes (Hakil et al. 2014). This polyphyletic diversity of alkane oxidizers within the *Desulfobacteraceae*, and their phylogenetically intertwined position among many aromatics-degrading strains and bacteria that do not degrade hydrocarbons

(Fig. 2), shows that the molecular detection of alkane oxidizers is not possible on the basis of 16S rRNA sequences alone or remains at the least ambiguous. Almost certainly, hydrocarbon oxidizers are hiding in plain sight among diverse 16S rRNA phylotypes of the *Desulfosarcina*/*Desulfococcus* group and other deltaproteobacterial lineages that are typically recovered from marine sediments (Lloyd et al. 2006; Klepac-Ceraj et al. 2004).

Long- and short-chain alkane oxidation is not limited to members of the *Desulfobacteraceae* (Fig. 3). The decane-oxidizer *Desulfothermus naphthae* (strain TD3) from Guaymas Basin forms a separate phylogenetic lineage that shares a root with *Desulfovibrio* spp. (Rüter et al. 1994). The *Firmicutes* include the propane-oxidizing thermophilic *Desulfotomaculum* strain Propane60-GuB from Guaymas Basin (Kniemeyer et al. 2007) and the phenol-, toluene-, and benzoate-oxidizing, sulfate- and arsenate-reducing *Desulfosporosinus* strain Y5 from freshwater lake sediments (Liu et al. 2004). The *Firmicutes* are likely to be involved in hydrocarbon oxidation; for example, sulfate reducers of the genus *Desulfotomaculum* are commonly isolated from a wide range of subsurface habitats, often with petroleum hydrocarbon sources (reviewed in Amend and Teske 2005). Two strains of the new genus and species *Desulfoglaeba alkanexedens*, isolated from an oily wastewater storage facility and from oil field production water, specialize in the complete oxidation of C_6–C_{12} *n*-alkanes and selected organic acids; these strains belong to the family *Syntrophobacteraceae* within the *Deltaproteobacteria* (Davidova et al. 2006).

7 Short-Chain Alkane-Oxidizing Consortia

Enrichments and pure culture isolation have solved the riddle of a deeply branching bacterial lineage that was originally found in clone library surveys of Guaymas Basin sediments (clones A1B020 and A2B002, Teske et al. 2002). This bacterial lineage appeared again in thermophilic enrichments at 60 °C from Guaymas Basin sediments where n-butane had been added as substrate (Kniemeyer et al. 2007). Subsequently, FISH analysis of thermophilic Guaymas Basin enrichments with methane and sulfate revealed that bacteria of this lineage – subsequently termed the HotSeep-1 group (Fig. 3) – formed syntrophic associations with ANME-1 archaea; the HotSeep-1 bacteria had obviously replaced the syntrophic *Desulfobacteraceae* that are commonly found at cool temperatures (Holler et al. 2011). In follow-up studies of this syntrophic association, the Hotseep-1 bacteria were discovered to form pili-like cell-to-cell conduits for direct electron exchange with their ANME-1 partners (Wegener et al. 2015). Subsequently, the HotSeep-1 bacteria were found growing in syntrophic association with butane-oxidizing archaea, the euryarchaeon *Candidatus* Syntrophoarchaeum spp. (Laso-Pérez et al. 2016); the syntrophic role of HotSeep-1 bacteria is to channel the electrons obtained by its butane-oxidizing archaeal partner to the terminal electron acceptor sulfate (see Sect. 10). While the archaeal butane oxidizers so far require co-culture with their

Hotseep-1 syntrophs, this bacterium was also isolated in pure culture and described as the thermophilic, hydrogenotrophic sulfate reducer *Candidatus* Desulfofervidus auxilii (Krukenberg et al. 2016); this is the first time that a sulfate-reducing bacterial syntroph of archaeal methane and alkane oxidizers could be isolated and maintained separately from its archaeal partner. It is possible that this ability to change from a free-living to a syntrophic, consortial lifestyle favors *Candidatus* Desulfofervidus auxilii in its natural habitat and explains the widespread occurrence of this bacterium in the hydrothermal sediments of Guaymas Basin; its 16S rRNA genes are consistently found in bacterial clone library surveys of diverse Guaymas Basin sediments (Dowell et al. 2016).

Exploring the physiology, genomics, and habitat preferences of sulfate-dependent, syntropic short-chain alkane-oxidizing consortia is work in progress; a greater range of seep habitats should be targeted and surveyed. For example, *Candidatus* Syntrophoarchaeum is not limited to Guaymas Basin; closely related 16S rRNA genes [but not those of *Candidatus Desulfofervidus auxilii*] have been found in brine seeps in the Gulf of Mexico (Genbank No. AM745246; Orcutt et al. 2010). It is also possible that *Candidatus* Desulfofervidus auxilii could be replaced by yet another type of alkane-oxidizing syntroph, to accommodate local thermal and geochemical regimes (Fig. 3).

The thermal range of bacterial/archaeal syntrophic consortia in Guaymas Basin that oxidize short-chain alkanes and methane is surprisingly broad; *Candidatus* Desulfofervidus auxilii formed methane-oxidizing consortia with ANME-1 archaea in enrichment cultures at 37 °C (Kellermann et al. 2012) and at 50 °C (Holler et al. 2011; Wegener et al. 2015), in addition to forming butane-oxidizing consortia at 60 °C (Kniemeyer et al. 2007; Laso-Pérez et al. 2016); the upper thermal range, at least for Hotseep-1/ANME-1 consortia, appears to be near 70 °C (Holler et al. 2011). Supporting evidence for a broad, mesophilic to thermophilic range of short-chain alkane and methane oxidation comes from biogeochemical field observations in Guaymas Basin. Extensive $\delta^{13}C$ measurements of pore water methane from the southern Guaymas vent field indicated a hydrothermal baseline near −42‰ for methane produced by pyrolysis of buried organic matter (McKay et al. 2016), similar to previous measurements (−43‰ to −51‰; Welhan 1988). More positive $\delta^{13}C$ values for methane begin to appear under the influence of microbial methane oxidation at temperatures below ca. 70–75 °C and persist throughout the moderately thermophilic and mesophilic range (McKay et al. 2016). Short-chain alkanes [ethane to hexane] show heavier $\delta^{13}C$ values, in the case of ethane reaching even positive values near +5‰ in a moderately heated sediment core with in situ temperatures below 40 °C; these results suggest the isotopic imprint of microbial oxidation that selectively removes isotopically lighter alkanes (Dowell et al. 2016). Most interestingly, the hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus* (type strain VC-16) was shown to grow on a wide range of alkenes (C_{12}–C_{21}, *n*-alk-1-enes) and fatty acids, as well as *n*-alkanes (C_{10}–C_{21}) at 70 °C, provided that the incubation times were extended to 1 to 2 months (Khelifi et al. 2010, 2014). A largely unexplored role for the Archaeoglobales in alkane degradation is further

supported by the intriguing observation that thermophilic methanogenic enrichments oxidizing long-chain alkanes in the absence of sulfate were dominated by members of the Archaeoglobales (Mbadinga et al. 2012).

8 Aromatics-Oxidizing Sulfate-Reducing Bacteria

Molecular monitoring of enrichments for aromatics-degrading, specifically alkylbenzene-oxidizing, sulfate reducers has shown that the sulfate-reducing communities that grow on these substrates in crude oil belong to the *Desulfobacteraceae*, a family of *Deltaproteobacteria* that remineralizes a wide range of carbon substrates completely to CO_2; members of the deltaproteobacterial order *Desulfovibrionales* constitute a minority in such enrichments (Rabus et al. 1996). These results are consistent with the well-known substrate range of cultured species; for example, the species *Desulfosarcina variabilis*, *Desulfobacterium cetonicum*, and *Desulfonema magnum* within the *Desulfobacteraceae* can oxidize benzoate, whereas members of the *Desulfovibrionales* do not (Widdel and Bak 1992). Cultivation efforts have augmented the number of new aromatics-oxidizing strains, species, and genera within the *Desulfobacteraceae*: the benzoate-, toluene-, and *o*-xylene-oxidizing strain *Desulfosarcina ovata* strain oXyS1 from a North Sea oil tank (Harms et al. 1999), the psychrophilic benzoate degrader *Desulfoconvexum algidum* from arctic sediments (Könneke et al. 2013), and *Desulfatirhabdium butyrativorans* from an anaerobic bioreactor which degrades various substituted aromatics (Balk et al. 2008). Within the *Desulfobacter* lineage, the phylotype SB-9 from a Guaymas Basin enrichment on benzene (Phelps et al. 1998) was most closely related to the toluene-, phenol-, or benzoate-oxidizing sulfate reducers *Desulfobacula toluolica* (Rabus et al. 1993), *Desulfobacula phenolica* (Bak and Widdel 1986), and *Desulfotignum balticum* (Küver et al. 2001) from anoxic marine sediments and to the toluene oxidizer *Desulfotignum toluenicum* from a crude oil reservoir model column (Ommedal and Torsvik 2007). Other phylotypes closely related to these cultured isolates were obtained from marine sediment habitats, such as mud volcanoes (Lösekann et al. 2007). To summarize, the *Desulfobacteraceae* are not only exceptionally rich in marine alkane-degrading isolates but in aromatics-degrading sulfate reducers as well (Fig. 3).

A sister lineage to the *Desulfobacteraceae*, the *Desulfatiglans* lineage, consists exclusively of aromatics-degrading sulfate-reducing bacteria that were isolated from a wide range of marine and estuarine sediments and hydrocarbon-processing facilities. Strain EbS7, capable of complete oxidation of ethylbenzene, was isolated from Guaymas Basin sediments (Kniemeyer et al. 2003). This strain is closely related to aromatics-oxidizing counterparts from other marine habitats, the naphthalene oxidizer Naph2S (Galushko et al. 1999) and the *m*-xylene oxidizing strain mXyS1 (Harms et al. 1999). Together, these three strains – which are not yet formally described as new species or genera – form a distinct deltaproteobacterial phylogenetic lineage that includes the two aromatics-degrading species *Desulfatiglans anilini*,

with two strains AK1 (Ahn et al. 2009) and DSM 4660^T, (Kniemeyer et al. 2003), and *Desulfatiglans parachlorophenolica* (Suzuki et al. 2014). A sediment-free mixed culture enriched from Guaymas Basin sediments with benzene as the sole substrate yielded two phylotypes (SB-21 and SB-30) within this lineage (Phelps et al. 1998). Phylotypes of the *Desulfatiglans* cluster are frequently and consistently recovered in 16S rRNA and functional gene (*dsr*AB) clone libraries from marine hydrocarbon seep sediments (Lloyd et al. 2006), marine surface-breaching methane hydrates (Mills et al. 2003; Mills et al. 2005), marine mud volcano sediments (Lösekann et al. 2007), deep marine subsurface sediments (Inagaki et al. 2006), but also estuarine sediments that are rich in plant biomass (Klepac-Ceraj et al. 2004, Bahr et al. 2005). The substrate preferences of the uncultured members of this cluster remain speculative (i.e., plant phenolic compounds) and have to be verified by further enrichment and isolation efforts. At present, the *Desulfatiglans* lineage is not included among the *Desulfobacteraceae* in the strict sense (Küver 2014); ultimately it may require description as a new family.

The wide spectrum of alkane- and aromatics-degrading sulfate-reducing isolates from hydrocarbon-rich vent sites and cold seep environments clearly shows that the sulfate-reducing community is a major player in the biodegradation of specific petroleum components in nature. Individual sulfate-reducing strains have quite specific substrate requirements and effect only a partial biotransformation of a petroleum sample by selectively removing specific alkanes and aromatics compounds (Bazylinski et al. 1988; Rüter et al. 1994). However, a diversified community of hydrocarbon-oxidizing specialists should be able to alter the alkane and aromatics spectrum of complex hydrocarbon mixtures in petroleum samples profoundly and also exploit synergies in substrate utilization. Hydrocarbon-degrading strains with different substrate specificities that grow in mixed culture and in natural enrichments can combine their degradative capabilities, even to the point of bridging pathway gaps in individual community members that would not be able to grow in isolation. For example, a mixed aerobic community from Gulf of Mexico surface water after the Deepwater Horizon oil spill was used for stable isotope probing experiments with ^{13}C-labeled phenanthrene, naphthalene, and hexadecane, followed by metagenomic analysis of the resulting enrichments. Unexpectedly, obligate alkane-degrading bacteria such as *Alcanivorax* appeared as dominant community members not only in the hexadecane-degrading enrichments but also in the naphthalene-degrading enrichments (Dombrowski et al. 2016). Although the naphthalene-enriched *Alcanivorax* population did not harbor complete aromatics-degrading pathways, this population remained sufficiently stable and abundant within the naphthalene-enriched community to contribute a high-quality genome (Dombrowski et al. 2016). These results suggest that hydrocarbon-oxidizing bacteria possessing incomplete hydrocarbon-degrading pathways for a particular substrate can persist in natural enrichments, as long as the microbial consortium as a whole carries the necessary genes and expresses the enzymes that are collectively needed to degrade, oxidize, and assimilate the available hydrocarbons.

Sulfate reduction by free-living or syntrophic microorganisms produces hydrogen sulfide and other reduced sulfur species; thus, sulfide is a common occurrence in petroleum reservoirs and hydrocarbon seeps. Its abundance favors the growth of sulfur-oxidizing bacteria as soon as suitable electron acceptors (oxygen, nitrate) become available, commonly by seawater inmixing, groundwater recharge, or drilling operations. For example, a 16S rRNA cloning and DGGE survey of bacterial communities in oil-contaminated groundwater yielded mostly epsilon-proteobacterial phylotypes related to the denitrifying and microaerophilic sulfide oxidizers *Sulfurimonas denitrificans* and *Arcobacter* spp. (Watanabe et al. 2000). A cultivation-based enrichment study of sulfur oxidizers from saline production water of Canadian oil fields yielded sulfur oxidizers related to the epsilon-proteobacterial genus *Arcobacter* and the gamma-proteobacterial genus *Thiomicrospira*, often in co-culture with sulfate-reducing bacteria of the genera *Desulfovibrio*, *Desulfomicrobium*, and *Desulfobulbus* and of the family *Desulfobacteraceae* (Voordouw et al. 1996). It is unlikely that these oxygen- and nitrate-respiring, sulfur- and sulfide-oxidizing bacteria survive within fully anoxic, reduced petroleum reservoirs; they would require some admixture of oxidants by groundwater or production water circulation. However, once oxygen and nitrate are supplied, these bacteria can regenerate sulfate by oxidizing hydrogen sulfide or other reduced sulfur species and provide the electron acceptor for sulfate-reducing populations that oxidize petroleum hydrocarbons (Voordouw et al. 1996).

9 Methanogenic Archaea and Microbial Alkane Cracking

Methanogenic enrichments and isolates are often obtained from hydrocarbon-rich sediments and deep oil reservoirs. Generally, methanogens reduce a limited spectrum of low-molecular-weight carbon substrates (CO_2/H_2, formate, acetate, methanol, and methyl groups, in some cases ethanol) to methane. Unlike sulfate-reducing bacteria, methanogens are not oxidizing petroleum hydrocarbons directly, but they occur consistently as community members of hydrocarbon-degrading microbial communities, where they play an essential role in complete degradation of alkanes by defined bacterial/archaeal consortia.

Pure culture isolations of methanogens from oil fields have often resulted in moderately thermophilic or thermophilic, CO_2/H_2-utilizing methanogens, in particular new species of the genera *Methanobacterium*, *Methanococcus*, and *Methanothermococcus* (reviewed in Magot et al. 2001; Dahle et al. 2008). The hydrothermally heated petroleum-rich sediments of Guaymas Basin have yielded hyperthermophilic, CO_2/H_2 autotrophs of the genus *Methanocaldococcus*, with optimal growth temperatures between 80 and 90 °C (Jeanthon et al. 1999; Jones et al. 1983, 1989). These repeated isolations of hydrogenotrophic methanogens suggest that hydrogen is an important methanogenic substrate in oil fields and hydrocarbon-rich sediments. Members and close relatives of the acetoclastic genus *Methanosaeta* are also consistently detected, either by pure culture isolation (Magot et al. 2000),

as enrichments (Zengler et al. 1999), or molecular phylotypes (Dhillon et al. 2005; Watanabe et al. 2002).

Strictly anaerobic, sulfate-free incubations with Guaymas Basin sediments yielded sediment-free stable methanogenic enrichments that were growing on hexadecane as sole substrate (Zengler et al. 1999). This oxidation of unbranched long-chain alkanes amounts to a strictly anaerobic microbial alkane cracking process that most likely proceeds via syntrophic anaerobic alkane degradation to acetate and hydrogen, coupled to acetoclastic and CO_2/H_2 methanogenesis (Parkes 1999; Zengler et al. 1999). Thus, the methanogen communities are performing the terminal methanogenic remineralization steps in a complex network of syntrophic hydrocarbon degradation pathways, where the alkane-activating reactions and its microbial catalysts remain to be identified. The methanogenic enrichments growing in the laboratory on hexadecane contain *Methanosaeta*- and *Methanoculleus*-related 16S rRNA phylotypes (Zengler et al. 1999) and show a similar hydrogen- and acetate-utilizing methanogen community as the Guaymas sediments themselves (Dhillon et al. 2005). Consistent with a hydrogen-utilizing methanogen community, most of the bacterial 16S rRNA gene sequences in the alkane cracking Guaymas enrichment were members of the deltaproteobacterial genus *Syntrophus*, which grows in syntrophic association with H_2-consuming methanogens. As demonstrated by laboratory enrichments at 28 °C, microbial alkane cracking does not require high temperatures (Zengler et al. 1999); this process could be widespread under mesophilic conditions. For example, methanogenic degradation of short-chain alkanes (C_6–C_{10}), monoaromatics, and intermediate-size alkanes (C_{14}, C_{16}, C_{18}) in oil sand tailings proceeded toward complete oxidation at ca. 20 °C (Siddique et al. 2007, 2011).

This model of methanogenic hydrocarbon degradation occurring predominantly via syntrophic oxidation of alkanes to acetate and hydrogen, followed by H_2/CO_2 and acetate-dependent methanogenesis, is consistent with results of several, mutually independent microbial community studies. In hydrocarbon degradation experiments using laboratory microcosms with crude oil from North Sea oil fields (Jones et al. 2008), the archaeal community was dominated by hydrogen-oxidizing methanogens (86–87% of archaeal 16S rRNA gene phylotypes), with a minority of acetate-utilizing methanogens (6–13% of archaeal 16S rRNA gene phylotypes); the bacterial community was again dominated by *Syntrophus* spp. This community composition and their ^{13}C-isotopic imprint on biogenic methane were consistent with the predicted δ^{13}-isotopic signature of biogenic methane and CO_2 pools in field studies (Jones et al. 2008). Similar results have been obtained for oil-contaminated groundwater at the bottom of an underground crude oil storage cavity in Japan (Watanabe et al. 2002). Here, ca. 50% of all archaeal phylotypes obtained with a wide range of archaeal 16S rRNA primers were members of the hydrogen-utilizing family *Methanomicrobiales*; 7% were nearly identical (99% 16S rRNA gene similarity) with the acetoclastic species *Methanosaeta concilii*, and 17% were nearly identical to the methylotrophic species *Methanomethylovorans hollandica* (Watanabe et al. 2002). The dominance of members of the *Methanomicrobiales*

has been confirmed in a 16S rRNA survey of offshore oil reservoirs in California (Orphan et al. 2000). Here, 86% of all archaeal phylotypes fell into the *Methanomicrobiales*, closely related to the methanogen *Methanoplanus petrolearius*, a species that was isolated from an oil well (Ollivier et al. 1997); 4% of all archaeal phylotypes were related to the *Methanosarcinales* (Orphan et al. 2000). There is some evidence that the spectrum of available hydrocarbons influences the types of methanogens that are enriched during syntrophic degradation. Adding the short-chain alkanes C_6–C_{10} to primary enrichments from oil sand tailings resulted in the enrichment of acetoclastic *Methanosaetaceae*, whereas adding the mixed monoaromatics benzene, toluene, ethylbenzene, and xylene resulted in the enrichment of hydrogenotrophic *Methanomicrobiales* (Siddique et al. 2012).

10 Bacterial and Archaeal Alkane Oxidizers

Until recently, a fundamental difficulty of the "microbial alkane cracking" model persisted: The organisms that anaerobically oxidize and assimilate the alkanes have not been explicitly identified, but were presumed to be bacteria by default since no alkane-oxidizing archaea were known, and methanogens were assigned a supporting role as syntrophs that consume hydrogen or acetate. Clone library sequencing, single-cell sequencing, and metagenomic analysis of syntrophic methanogenic enrichments had consistently yielded *Deltaproteobacteria* related to the genera *Syntrophus* and *Smithella* (Zengler et al. 1999; Gray et al. 2011; Siddique et al. 2012; Embree et al. 2014). Considering the evidence, the critical alkane-oxidizing community members could be members of the genera *Syntrophus* and *Smithella*, a possibility that remains consistent with genome annotation of *Smithella* (Tan et al. 2014); alternatively, alkane-oxidizing archaea hiding in plain sight could have been mistaken for methanogens or remained concealed within the "unidentified" background. It turned out that these possibilities do not exclude each other.

Metagenomic sequencing and gene expression analysis of a long-chain *n*-alkane (paraffins, C_{28}–C_{50})-oxidizing consortium have so far provided the strongest evidence that bacteria related to the genera *Syntrophus* and *Smithella* are the key players (Wawrik et al. 2016). Dominant community members of this paraffin-oxidizing consortium include bacteria of the fermentative genus *Smithella* and the sulfur-reducing genus *Desulfuromonas*, and methanogenic archaea of the hydrogenotrophic genera *Methanoculleus* and *Methanolinea*, and the acetoclastic genus *Methanosaeta* (Wawrik et al. 2016). The key enzyme alkylsuccinate synthase that catalyzes anaerobic alkane activation via fumarate addition was found in the *Smithella* genome and was also expressed during paraffin degradation. Metagenomic reconstruction supports the following scenario for the syntrophic *Smithella* strain: Electrons derived from beta-oxidation of the activated alkanes undergo reverse electron transfer to produce hydrogen or formate, possibly via an electron transfer flavoprotein to a membrane-bound FeS oxidoreductase, and from

there to a periplasmatic formate dehydrogenase/hydrogenase. Acetate produced after beta-oxidation from acetyl-CoA is excreted and converted to CO_2 and hydrogen by the syntrophic *Desulfuromonas* strain. These endergonic hydrogen- and acetate-producing reactions are then coupled to exergonic hydrogenotrophic or acetoclastic methanogenesis, to drive the combined reaction of methanogenic paraffin oxidation to CO_2 (Wawrik et al. 2016). The *Syntrophus* strain forms a sister lineage to the cultured *Syntrophus* species *S. gentianae*, *S. buswellii*, and *S. aciditrophicus* and is most closely related to *Smithella propionica* (Wawrik et al. 2016), a fatty acid-oxidizing fermentative bacterium that converts propionate to acetate, CO_2, and methane in syntrophic methanogenic co-culture (Liu et al. 1999).

The euryarchaeotal candidate genus *Syntrophoarchaeum* that completely oxidizes *n*-butane and propane provides the best example for archaea as the primary agents of short-chain alkane oxidation. This archaeon and its HotSeep-1 bacterial syntroph were enriched from Guaymas Basin sediments on *n*-butane, selected by dilution to extinction, and finally maintained in sediment-free minimal medium with *n*-butane as sole carbon substrate at 50 °C (Laso-Pérez et al. 2016). *Syntrophoarchaeum* spp. use modified types of methane-coenzyme M reductase for butane activation; previously, *mcr*A genes were known as the key gene of methane activation in anaerobic methane oxidation and – in the reductive direction – of reducing the cofactor-bound methyl group to methane (Laso-Pérez et al. 2016). The butane-activating *mcr*A variant of *Syntrophoarchaeum* is related to other *mcr*A forms found in members of the *Bathyarchaeota*, indicating lateral gene transfer of *mcr*A genes between the Euryarchaeota and the *Bathyarchaeota*, two different archaeal phylum-level lineages (Evans et al. 2015). Recently, metagenomic screening of Guaymas Basin sediments detected novel *mcr*A gene versions related to the gene variant of *Syntrophoarchaeum*, but hosted by an archaeon of the GoM-Arc-1 cluster within the Euryarchaeota (Dombrowski et al. 2017). Novel types of *mcrA* genes are also associated with recently discovered lineages of methanogenic archaea, such as the cultured *Methanomassiliicoccales* (Borrel et al. 2014), the *Candidatus* lineage *Methanofastidiosales* (Nobu et al. 2016), and the candidate phylum Verstraetearchaeota (Vanwonterghem et al. 2016).

Exploring the physiology, genomics, and habitat range of short-chain alkane-oxidizing archaea is work in progress. The recent detection of entirely novel linages of *mcr*A genes in Guaymas Basin, so far not assigned to a specific microorganism, indicates the existence of further, yet unknown methane−/alkane-oxidizing archaea (Lever and Teske 2015). New *mcr*A gene types related to those found previously in members of the archaeal phylum *Bathyarchaeota* (Evans et al. 2015) have been detected in hot springs of Yellowstone National Park (McKay et al. 2017). While the identity of these archaea is not known with certainty, the *mcr*A gene phylogeny would be consistent with new types of *Bathyarchaeota* (McKay et al. 2017). The physiological function and genomic context of new *mcr*A gene variants remain to be

explored by enrichment, isolation, and [meta]genomic analysis of these archaeal candidates for methane and alkane oxidation.

11 Research Needs

Our presently sketchy understanding of hydrocarbon substrate utilization, microbial physiology, and ecosystem function at Guaymas Basin, in the Gulf of Mexico, on subsurface hydrocarbon reservoirs, and in other microbial habitats with a significant hydrocarbon imprint would benefit most from the enrichment, pure-culture isolation, and physiological study of not-yet-cultured bacteria and archaea. On top of the wish list, one might put the pure culture isolation of anaerobic, methane-oxidizing archaea, or the consortia that they are forming with sulfate-reducing bacteria. Methane-oxidizing archaea are so consistently associated with hydrocarbon seeps of all flavors that they can be regarded as the signature organisms of these environments (Knittel et al. 2005). In the absence of pure cultures or pure consortia, basic questions about these archaea remain unanswered or remain at the stage of interesting working scenarios: the links between gene presence and actual expression and regulation of methane-producing vs methane-oxidizing pathways (Hallam et al. 2004) or the physiological basis for novel, multiple bacterial symbioses involving methane-oxidizing archaea (Pernthaler et al. 2008). High on the wish list would be the identification of bacteria and archaea that are capable of microbial ethanogenesis and propanogenesis, thermodynamically feasible biologically processes that take place in organic-rich, methanogenic marine subsurface sediments (Hinrichs et al. 2006). On the level of biochemistry and cell physiology, the genetic diversity, enzymatic function, and environmental imprint of novel anaerobic hydrocarbon degradation pathways are an inexhaustible research field that benefits enormously from the availability of diverse hydrocarbon-oxidizing sulfate-reducing bacteria as model systems (Widdel and Rabus 2001; Muyzer and van der Kraan 2008). The identification of key metabolites in environmental samples will help to link pure culture studies and field results on microbial community composition and activity (Gieg and Suflita 2002). This approach would be particularly powerful when combined with metagenomic exploration of natural hydrocarbon-degrading communities and enrichments, to identify novel community members and their genomic blueprint (Dombrowski et al. 2017). New detection methods for active hydrocarbon-degrading cells and populations – such as the BONCAT approach that is already proving highly useful for methane-cycling archaea – would open up new avenues to explore chemical and physical stimuli that influence cellular activity (Hatzenpichler et al. 2016). Finally, the isolation and biochemical study of anaerobic, thermophilic archaeal alkane oxidizers will elucidate the high-temperature range of alkane oxidation and assimilation, a field of particular interest due to the elevated temperatures in many hydrocarbon reservoirs.

Acknowledgments Andreas Teske was supported by NSF (Biological Oceanography 0647633 and 1357238 for Guaymas Basin; Emerging Frontiers/Microbial Observatories: Microbial Interactions and Processes 0801741 for the Gulf of Mexico) and by the Gulf of Mexico Research Institute [GOMRI] through the ECOGIG [Ecological Consequences of Oil and Gas input into the Gulf of Mexico] consortium.

References

Aeckersberg F, Bak F, Widdel F (1991) Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. Arch Microbiol 156:5–14

Aeckersberg F, Rainey F, Widdel F (1998) Growth, natural relationships, cellular fatty acids and metabolic adaptation of sulfate-reducing bacteria that utilize long-chain alkanes under anoxic conditions. Arch Microbiol 170:361–369

Ahn Y-B, Chae J-C, Zylstra GJ, Häggblom MM (2009) Degradation of phenol via phenylphosphate and carboxylation to 4- hydroxybenzoate by a newly isolated strain of the sulfate-reducing bacterium *Desulfobacterium anilini*. Appl Environ Microbiol 75:4248–4253

Amend JP, Teske A (2005) Expanding frontiers in deep subsurface microbiology. Palaeogeogr Palaeoclimatol Palaeoecol 219:131–155

Amos S (1987) Late Triassic-Jurassic paleogeography and origin of Gulf of Mexico Basin. AAPG Bull 71(4):419–451

Bahr M, Crump BC, Klepac-Ceraj V, Teske A, Sogin ML, Hobbie JE (2005) Molecular characterization of sulfate-reducing bacteria in a New England salt marsh. Environ Microbiol 7:1175–1185

Bak F, Widdel F (1986) Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum*. Arch Microbiol 146:177–180

Balk M, Altinbas M, Rijpstra WIC, Sinninghe Damste JS, Stams AJM (2008) Desulfatirhabdium butyrativorans gen nov., sp. nov., a butyrate-oxidizing, sulfate-reducing bacterium isolated from an anaerobic bioreactor. Int J Syst Evol Microbiol 58:110–115

Bazylinski DA, Farrington JW, Jannasch HW (1988) Hydrocarbons in surface sediments from a Guaymas Basin hydrothermal vent site. Org Geochem 12:547–558

Beeder J, Nilsen RK, Rosnes JT, Torsvik T, Lien T (1994) *Archaeoglobus fulgidus* isolated from hot north sea oil field waters. Appl Environ Microbiol 60:1227–1231

Beeder J, Torsvik T, Lien T (1995) *Thermodesulforhabdus norvegicus* gen. nov., sp. nov., a novel thermophilic sulfate-reducing bacterium from oil field water. Arch Microbiol 164:331–336

Berndt C, Hensen C, Mortera-Gutierrez C, Sarkar S, Geilert S, Schmidt M, Liebetrau V, Kipfer R, Scholz F, Doll M, Muff S, Karstens J, Planke S, Petersen S, Böttner C, Chi W-C, Moser M, Behrendt R, Fiskal A, Lever MA, Su C-C, Deng L, Brennwald MS, Lizarralde D (2016) Rifting under steam – how magmatism triggers methane venting from sedimentary basins. Geology 44:767–770

Biddle JF, Lipp JS, Lever M, Lloyd K, Sørensen K, Anderson R, Fredricks HF, Elvert M, Kelly TJ, Schrag DP, Sogin ML, Brenchley JE, Teske A, House CH, Hinrichs K-U (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. Proc Natl Acad Sci U S A 103:3846–3851

Biddle JF, Cardman Z, Mendlovitz H, Albert DB, Lloyd KG, Boetius A, Teske A (2012) Anaerobic oxidation of methane at different temperature regimes in Guaymas Basin hydrothermal sediments. ISME J 6:1018–1031

Borrel G, Parisot N, Harris HM, Peyretaillade E, Gaci N, Tottey W, Bardot O, Raymann K, Gribaldo S, Peyret P, O'Toole PW, Brugère JF (2014) Comparative genomics highlights the unique biology of Methanomassiliicoccales, a Thermoplasmatales-related seventh order of methanogenic archaea that encodes pyrrolysine. BMC Genomics 15:1–23

Bowles MW, Samarkin VA, Bowles K, Joye SB (2011) Weak coupling between sulfate reduction and the anaerobic oxidation of methane in methane-rich seafloor sediments during ex-situ incubation. Geochim Cosmochim Acta 75:500–519

Brooks JM, Kennicutt MC, Fay RR, McDonald TJ, Sassen R (1984) Thermogenic gas hydrates in the Gulf of Mexico. Science 223:696–698

Brooks JM, Cox HB, Bryan WR, Kennicutt MC II, Mann RG, MacDonald TJ (1986) Association of gas hydrates and oil seepage in the Gulf of Mexico. Org Geochem 10:221–234

Bryant RW, Bryant JR, Feeley MH, Simmons GR (1990) Physiographic and bathymetric characteristics of the continental slope, Northwest Gulf of Mexico. Geo-Mar Lett 10:182–199

Calvert SE (1966) Origin of diatom-rich varved sediments from the Gulf of California. J Geol 76:546–565

Cravo-Laureau C, Matheron R, Cayol J-L, Joulian C, Hirschler-Réa A (2004a) *Desulfatibacillum aliphaticivorans* gen. nov., sp. nov., an *n*-alkane- and *n*-alkene-degrading, sulfate-reducing bacterium. Int J Syst Evol Microbiol 54:77–83

Cravo-Laureau C, Matheron R, Joulian C, Cayol J-L, Hirschler-Réa A (2004b) *Desulfatibacillum alkenivorans* sp. nov., a novel *n*-alkene-degrading, sulfate-reducing bacterium, and emendated description of the genus *Desulfatibacillum*. Int J Syst Evol Microbiol 54:1639–1642

Cravo-Laureau C, Labat C, Joulian C, Matheron R, Hirschler-Réa A (2007) *Desulfatiferula oiefinivorans* gen. nov, sp. nov. a long-chain *n*-alkane-degrading, sulfate-reducing bacterium. Int J Syst Evol Microbiol 57:2699–2702

Dahle H, Garshol F, Madsen M, Birjeland N-K (2008) Microbial community structure of produced water from a high-temperature North Sea oil-field. Antonie Van Leeuwenhoek 93:37–49

Davey ME, Wood WA, Key R, Nakamura K, Stahl DA (1993) Isolation of three species of *Geotoga* and *Petrotoga*: two new genera, representing a new lineage in the bacterial line of descent distantly related to the "Thermotogales". Syst Appl Microbiol 16:191–200

Davidova IA, Duncan KE, Choi OK, Suflita JM (2006) *Desulfoglaeba alkanexedens* gen. nov., sp. nov., an n-alkane-degrading, sulfate-reducing bacterium. Int J Syst Evol Microbiol 56:2737–2742

De la Lanza-Espino G, Soto LA (1999) Sedimentary geochemistry of hydrothermal vents in Guaymas Basin, Gulf of California, Mexico. Appl Geochem 14:499–510

Dhillon A, Teske A, Dillon J, Stahl DA, Sogin ML (2003) Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. Appl Environ Microbiol 69:2765–2772

Dhillon A, Lever M, Lloyd K, Albert DB, Sogin ML, Teske A (2005) Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (mcrA) genes (*mcr*A) in hydrothermal sediments of the Guaymas Basin. Appl Environ Microbiol 71:4592–4601

Didyk BM, Simoneit BR (1989) Hydrothermal oil of Guaymas Basin and implications for petroleum formation mechanisms. Nature 342:65–69

Dojka M, Hugenholtz P, Haack SK, Pace NR (1998) Microbial diversity in a hydrocarbon- and chlorinated solvent-contaminated aquifer undergoing intrinsic bioremediation. Appl Environ Microbiol 64:3868–3877

Dombrowski N, Donaho J, Gutierrez T, Teske AP, Baker BJ (2016) Metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill. Nat Microbiol 1:16057

Dombrowski N, Seitz K, Teske A, Baker B (2017) Genomic insights into potential interdependencies in microbial hydrocarbon and nutrient cycling in hydrothermal sediment communities. Microbiome 5:106. https://doi.org/10.1186/s40168-017-0322-2

Dowell F, Cardman Z, Dasarathy S, Kellermann MY, McKay LJ, MacGregor BJ, Ruff SE, Biddle JF, Lloyd KG, Lipp JS, Hinrichs K-U, Albert DB, Mendlovitz H, Teske A (2016) Microbial communities in methane and short alkane-rich hydrothermal sediments of Guaymas Basin. Front Microbiol 7:17. https://doi.org/10.3389/fmicb.2016.00017

Edgcomb V, Kysela D, Teske A, de Vera Gomez A, Sogin ML (2002) Benthic eukaryotic diversity in the Guaymas Basin, a hydrothermal vent environment. Proc Natl Acad Sci U S A 99:7658–7662

Einsele G, Gieskes JM, Curray J, Moore D, Aguayo E, Aubry MP et al (1980) Intrusion of basaltic sills into highly porous sediments and resulting hydrothermal activity. Nature 283:441–445

Elsgaard L, Isaksen MF, Jørgensen BB, Alayse A-M, Jannasch HW (1994) Microbial sulfate reduction in deep-sea sediments at the Guaymas Basin hydrothermal vent area: influence of temperature and substrates. Geochim Cosmochim Acta 58:3335–3343

Embree M, Nagarajan H, Movahedi N, Chitsaz H, Zengler K (2014) Single-cell genome and metatranscriptome sequencing reveal metabolic interactions of an alkane-degrading methanogenic community. ISME J 8:757–767

Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD, Tyson GW (2015) Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. Science 350:434–438

Fisher AT, Becker K (1991) Heat flow, hydrothermal circulation and basalt intrusions in the Guaymas Basin, Gulf of California. Earth Planet Sci Lett 103:84–89

Galimov EM, Simoneit BRT (1982) Geochemistry of interstitial gases in sedimentary deposits of the Gulf of California, deep sea drilling project leg 64. In: Curray JR, Blakeslee J, Platt LW, Stout LN, Moore DG, Aguayo JE et al (eds) Initial reports of the deep sea drilling project, vol 64. U.S. Government Printing Office, Washington, DC, pp 781–787

Galushko A, Minz D, Schink B, Widdel F (1999) Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. Environ Microbiol 1:415–420

Garcia-Pineda O, MacDonald I, Zimmer B, Shedd B, Roberts H (2010) Remote- sensing evaluation of geophysical anomaly sites in the outer continental slope, northern Gulf of Mexico. Deep-Sea Res II Top Stud Oceanogr 57:1859–1869

Giebel HA, Klotz F, Voget S, Poehlein A, Grosser K, Teske A, Brinkhoff T (2016) Draft genome sequence of the marine *Rhodobacteraceae* strain O3.65, cultivated from oil-polluted seawater of the Deepwater Horizon oil spill. Stand Genomic Sci 11:81. https://doi.org/10.1186/s407893-016-0201-7

Gieg L, Suflita JM (2002) Detection of anaerobic metabolites of saturated and aromatic hydrocarbons in petroleum-contaminated aquifers. Environ Sci Technol 36:3755–3762

Gieskes JM, Kastner M, Einsele G, Kelts K, Niemitz J (1982) Hydrothermal activity in the Guaymas Basin, Gulf of California: a synthesis. In: Curray JR, Blakeslee J, Platt LW, Stout LN, Moore DG, Aguayo JE et al (eds) Initial reports of the deep sea drilling project, vol 64. U.S. Government Printing Office, Washington, DC, pp 1159–1167

Götz FE, Jannasch HW (1993) Aromatic hydrocarbon-degrading bacteria in the petroleum-rich sediments of Guaymas Basin hydrothermal vent site: preference for aromatic carboxylic acids. Geomicrobiol J 11:1–18

Grassia GS, McLean KM, Glenat P, Bauld J, Sheehy AJ (1996) A systematic survey for thermophilic fermentative bacteria and archaea in high temperature petroleum reservoirs. FEMS Microbiol Ecol 21:47–58

Gray ND, Sherry A, Grant RJ, Rowan AK, Hubert CRJ, Callbeck CM, Aitken CM, Jones DM, Adams JJ, Larter SR, Head IM (2011) The quantitative significance of *Syntrophaceae* and syntrophic partnerships in methanogenic degradation of crude oil alkanes. Environ Microbiol 13:2957–2975

Greene AD, Patel BKC, Sheehy AJ (1997) *Deferribacter thermophilus* gen. nov., sp. nov., a novel thermophilic manganese- and iron-reducing bacterium isolated from a petroleum reservoir. Int J Syst Bacteriol 47:505–509

Guezennec JG, Dussauze J, Bian M, Rocchicioli F, Ringelberg D, Hedrick DB, White DC (1996) Bacterial community structure from Guaymas Basin, Gulf of California, as determined by analysis of phospholipid ester-linked fatty acids. J Mar Biotechnol 4:165–175

Gundersen JK, Jørgensen BB, Larsen E, Jannasch HW (1992) Mats of giant sulfur bacteria on deep-sea sediments due to fluctuation hydrothermal flow. Nature 360:454–455

Gutierrez T, Singleton DR, Berry D, Yang T, Aitken MD, Teske A (2013) Hydrocarbon-degrading bacteria enriched by the Deepwater Horizon oil spill identified by cultivation and DNA-SIP. ISME J 7:2091–2104

Hakil F, Amin-Ali O, Hirschler-Réa A, Mollex D, Grossi V, Duran R, Matheron R, Cravo-Laureau C (2014) Desulfatiferula berrensis sp. nov., a n-alkene-degrading sulfate-reducing bacterium isolated from estuarine sediments. Int J Syst Evol Microbiol 64:540–544

Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, Richardson PM, DeLong EF (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. Science 305:1457–1462

Harms G, Zengler K, Rabus R, Aeckersberg F, Minz D, RosselloMora R, Widdel F (1999) Anaerobic oxidation of o-xylene, m-xylene, and homologous alkylbenzes by new types of sulfate-reducing bacteria. Appl Environ Microbiol 65:999–1004

Hatzenpichler R, Connon S, Goudeau D, Malmstrom RR, Woyke T, Orphan VJ (2016) Visualizing in situ translational activity for identifying and sorting slow-growing archaeal-bacterial consortia. Proc Natl Acad Sci U S A 113:E4069–E4078

Hinrichs K-U, Hayes JM, Bach W, Spivack AJ, Hmelo LR, Holm NG, Johnson CG, Sylva SP (2006) Biological formation of ethane and propane in the deep marine subsurface. Proc Acad Natl Acad Sci USA 103:14684–14689

Holler T, Widdel F, Knittel K, Amann R, Kellermann MY, Hinrichs K-U, Teske A, Boetius A (2011) Thermophilic anaerobic oxidation of methane by marine microbial consortia. ISME J 5:1946–1956

Inagaki F, Nunoura T, Nakagawa S, Teske A, Lever MA, Lauer A, Suzuki M, Takai K, Delwiche M, Colwell FS, Nealson KH, Horikoshi K, D'Hondt SL, Jørgensen BB (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. Proc Natl Acad Sci U S A 103:2815–2820

Jaekel U, Musat N, Adam B, Kuypers M, Grundmann O, Musat F (2013) Anaerobic degradation of propane and butane by sulfate-reducing bacteria enriched from marine hydrocarbon cold seeps. ISME J 7:885–895

Jannasch HW, Nelson DC, Wirsen CO (1989) Massive natural occurrence of unusually large bacteria (*Beggiatoa* spp.) at a hydrothermal deep-sea vent site. Nature 342:834–836

Jeanthon C, Reysenbach AL, L'Haridon S, Gambacorta A, Pace NR, Glénat P, Prieur D (1995) *Thermotoga subterranea* sp. nov., a new thermophilic bacterium isolated from a continental oil reservoir. Arch Microbiol 164:91–97

Jeanthon C, L'Haridon S, Pradel N, Prieur D (1999) Rapid identification of hyperthermophilic methanococci isolated from deep-sea hydrothermal vents. Int J Syst Bacteriol 49:591–594

Johansen C, Todd AC, MacDonald IR (2017) Time series video analysis of bubble release processes at natural hydrocarbon seeps in the northern Gulf of Mexico. Mar Pet Geol 82:21–34

Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS (1983) *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch Microbiol 136:254–261

Jones WJ, Stugard CE, Jannasch HW (1989) Comparison of thermophilic methanogens from submarine hydrothermal vents. Arch Microbiol 151:314–319

Jones DM, Head IM, Gray ND, Adams JJ, Rowan AK, Aitken CM, Bennett B, Huang H, Brown A, Bowler BFJ, Oldenburg T, Erdmann M, Larter SR (2008) Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. Nature 451:176–180

Jørgensen BB, Boetius A (2007) Feast and famine – microbial life in the deep-sea bed. Nat Rev Microbiol 5:770–781

Jørgensen BB, Zawacki LX, Jannasch HW (1990) Thermophilic bacterial sulfate reduction in deep-sea sediments at the Guaymas Basin hydrothermal vents (Gulf of California). Deep-Sea Res I 37:695–710

Jørgensen BB, Isaksen MF, Jannasch HW (1992) Bacterial sulfate reduction above 100°C in deep-sea hydrothermal vent systems. Science 258:1756–1757

Joye SB, MacDonald IR, Montoya JP, Peccini M (2005) Geophysical and geochemical signatures of Gulf of Mexico seafloor brines. Biogeosciences 2:295–309

Joye SB, Samarkin VA, Orcutt BN, MacDonald IR, Hinrichs K-U, Elvert M, Teske A, Lloyd KG, Lever MA, Montoya JP, Meile CD (2009) Metabolic variability in seafloor brines revealed by carbon and sulphur dynamics. Nat Geosci 2:349–354

Kallmeyer J, Boetius A (2004) Effects of temperature and pressure on sulfate reduction and anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. Appl Environ Microbiol 70:1231–1233

Kallmeyer J, Ferdelman TG, Jansen K-H, Jørgensen BB (2003) A high pressure thermal gradient block for investigating microbial activity in multiple deep-sea samples. J Microbiol Methods 55:165–172

Kastner M (1982) Evidence for two distinct hydrothermal systems in the Guaymas Basin. In: Curray JR, Blakeslee J, Platt LW, Stout LN, Moore DG, Aguayo JE et al (eds) Initial reports of the deep sea drilling project. U.S. Government Printing Office, Washington, DC, pp 1143–1158

Kawka OE, Simoneit BRT (1987) Survey of hydrothermally generated petroleums from the Guaymas Basin spreading center. Org Geochem 11:311–328

Kellermann MY, Wegener G, Elvert M, Yoshinaga MY, Lin Y-S, Holler T, Mollar XP, Knittel K, Hinrichs K-U (2012) Autotrophy as predominant mode of carbon fixation in thermophilic anaerobic methane-oxidizing microbial communities. Proc Natl Acad Sci U S A 109:19321–19326

Kelley DS, Baross JA, Delaney RJ (2002) Volcanoes, fluids, and life at mid-ocean ridge spreading centers. Annu Rev Earth Planet Sci 30:385–491

Kennicutt MC, Brooks JM, Denoux GJ (1988) Leakage of deep, reservoired petroleum to the near surface on the Gulf of Mexico continental slope. Mar Chem 24:39–59

Khelifi N, Grossi V, Dolla A, Hamdi M, Tholozan JL, Ollivier B et al (2010) Anaerobic oxidation of fatty acids and alkenes by the hyperthemophilic sulfate-reducing archaeon, *Archaeoglobus fulgidus*. Appl Environ Microbiol 76:3057–3060

Khelifi N, Ali OA, Roche P, Grossi V, Brochier-Armanet C, Valette O, Ollivier B, Dolla A, Hirschler-Réa A (2014) Anaerobic oxidation of long-chain n-alkanes by the hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus*. ISME J 8:2153–2166

Kimes NE, Callaghan AV, Aktas DF, Smith WL, Sunner J, Golding BT, Drozdowska M, Hazen TC, Suflita J, Morris PJ (2013) Genomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. Front Microbiol 4:50. https://doi.org/10.3389/fmicb.2013.00050

Kleindienst S, Ramette A, Amann R, Knittel K (2012) Distribution and in situ abundance of sulfate-reducing bacteria in diverse marine hydrocarbon seep sediments. Environ Microbiol 14:2689–2710

Kleindienst S, Herbst FA, Stagars M, von Netzer F, von Bergen M, Seifert J, Peplies J, Amann R, Musat F, Lueders T, Knittel K (2014) Diverse sulfate-reducing bacteria of the *Desulfosarcina/Desulfococcus* clade are the key alkane degraders at marine seeps. ISME J 8:2029–2044

Klepac-Ceraj V, Bahr M, Crump BC, Teske AP, Hobbie JE, Polz MF (2004) High overall diversity and dominance of microdiverse relationships in salt marsh sulphate-reducing bacteria. Environ Microbiol 6:386–398

Klotz F, Brinkhoff T, Freeze H, Wietz M, Teske A, Simon M, Giebel H-A (2018) *Tritonibacter horizontis*, gen. nov., sp. nov., a member of the *Rhodobacteraceae*, isolated from the Deepwater Horizon oil spill. Int J Syst Evol Microbiol 68:736–744

Kniemeyer O, Fischer T, Wilkes H, Glöckner FO, Widdel F (2003) Anaerobic degradation of ethylbenzene by a new type of marine sulfate-reducing bacterium. Appl Environ Microbiol 69:760–768

Kniemeyer O, Musat F, Sievert SM, Knittel K, Wilkes H, Blumenberg M, Michaelis W, Classen A, Bolm C, Joye SB, Widdel F (2007) Anaerobic oxidation of short-chain hydrocarbons by marine sulphate-reducing bacteria. Nature 449:898–901

Knittel K, Boetius A, Lemke A, Eilers H, Lochte K, Pfannkuche O, Linke P, Amann R (2003) Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia Margin, OR). Geomicrobiol J 20:269–294

Knittel K, Lösekann T, Boetius A, Kort R, Amann R (2005) Diversity and distribution of methanotrophic archaea at cold seeps. Appl Environ Microbiol 71:467–479

Könneke M, Küver J, Galushko A, Jørgensen BB (2013) Desulfoconvexum algidum gen. nov., sp. nov. a psychrophilic sulfate-reducing bacterium isolated from permanently cold, marine sediment (Svalbard). Int J Syst Evol Microbiol 63:959–964

Krukenberg V, Harding K, Richter M, Glöckner F-O, Gruber-Vodicka HR, Adam B, Berg J, Knittel K, Tegetmeyer HE, Boetius A, Wegener G (2016) *Candidatus* Desulfofervidus auxilii, a hydrogenotrophic sulfate-reducing bacterium involved in the thermophilic anaerobic oxidation of methane. Environ Microbiol 18:3073–3091

Küver J (2014) The family Desulfobacteraceae. In: Rosenberg E et al (eds) The prokaryotes – deltaproteobacteria and epsilonproteobacteria. Springer, Berlin/Heidelberg, pp 45–73. https://doi.org/10.1007/978-3-642-39044-9_266

Küver J, Könneke M, Galushko A, Drzyzga O (2001) Reclassification of *Desulfobacterium phenolicum* and *Desulfobacula phenolica* comb. nov. and description of strain SaxT as *Desulfotignum balticum* gen. nov. sp. nov. Int J Syst Evol Microbiol 51:171–177

L'Haridon S, Reysenbach A-L, Glenat P, Prieur D, Jeanthon C (1995) Hot subterranean biosphere in a continental oil reservoir. Nature 377:223–224

Lanoil BD, Sassen R, La Duc MT, Sweet ST, Nealson KH (2001) Bacteria and Archaea physically associated with Gulf of Mexico gas hydrates. Appl Environ Microbiol 67:5143–5153

Lapham LL, Chanton JP, Martens CS, Sleeper K, Woolsey JR (2008) Microbial activity in surficial sediments overlying acoustic wipeout zones at a Gulf of Mexico cold seep. Geochem Geophys Geosyst 9:Q06001. https://doi.org/10.1029/2008GC001944

Laso-Pérez R, Wegener G, Knittel K, Widdel F, Harding KJ, Krukenberg V, Meier DV, Richter M, Tegetmeyer HE, Riedel D, Richnow H-H, Adrian L, Reemtsma T, Lechtenfeld O, Musat F (2016) Thermophilic archaea activate butane via alkyl-coenzyme M formation. Nature 539:396–401

Lerche I, Petersen K (1995) Salt and sediment dynamics. CRC Press, Boca Raton, 322 pages

Lever MA, Teske A (2015) Methane-cycling archaeal diversity in hydrothermal sediment investigated by general and group-specific functional gene and 16S rRNA gene PCR primers. Appl Environ Microbiol 81:1426–1441

Lin Y-S, Koch BP, Feseker T, Ziervogel K, Goldhammer T, Schmidt F, Witt M, Kellermann M, Zabel M, Teske A, Hinrichs K-U (2017) Near-surface heating of young rift sediment causes mass production and discharge of reactive dissolved organic matter. Sci Rep 7:44864. https://doi.org/10.1038/srep44864

Liu Y, Balkwill DL, Aldrich HC, Drake GR, Boone DR (1999) Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolinii*. Int J Syst Evol Bacteriol 49:545–556

Liu A, Garcia-Dominguez E, Rhine ED, Young LY (2004) A novel arsenate-respiring isolate that can utilize aromatic substrates. FEMS Microbiol Ecol 48:323–332

Lizarralde D, Soule A, Seewald J, Proskurowski G (2011) Carbon release by off-axis magmatism in a young sedimented spreading centre. Nat Geosci 4:50–54

Lloyd KG, Lapham L, Teske A (2006) An anaerobic methane-oxidizing community of ANME-1 archaea in hypersaline Gulf of Mexico sediments. Appl Environ Microbiol 72:7218–7230

Lloyd KG, Albert D, Biddle JF, Chanton L, Pizarro O, Teske A (2010) Spatial structure and activity of sedimentary microbial communities underlying a *Beggiatoa* spp. mat in a Gulf of Mexico hydrocarbon seep. PLoS One 5(1):e8738. https://doi.org/10.1371/journal.pone.0008738

Lonsdale P, Becker K (1985) Hydrothermal plumes, hot springs, and conductive heat flow in the Southern Trough of Guaymas Basin. Earth Planet Sci Lett 73:211–225

Lösekann T, Knittel K, Nadalig T, Fuchs B, Niemann H, Boetius A, Amann R (2007) Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano, Barents Sea. Appl Environ Microbiol 73:3348–3362

MacDonald IR, Peccini MB (2009) Distinct activity phases during the recent geological history of a Gulf of Mexico mud volcano. Mar Pet Geol 26:1824–1830

MacDonald IR, Reilly FJ, Guinasso JNL, Brooks JM, Carney RS, Bryant WA, Bright TJ (1990) Chemosynthetic mussels at a brine-filled pockmark in the northern Gulf of Mexico. Science 248:1096–1099

MacDonald IR, Buthman DB, Sager WW, Peccini MB, Guinasso NL (2000) Pulsed oil discharge from a mud volcano. Geology 28:907–910

Magot M, Ollivier B, Patel BC (2000) Microbiology of petroleum reservoirs. Antonie Van Leeuwenhoek 77:103–116

Martens CS (1990) Generation of short chain organic acid anions in hydrothermally altered sediments of the Guaymas Basin, Gulf of California. Appl Geochem 5:71–76

Mbadinga SM, Li K-P, Zhou L, Wang L-Y, Yang S-Z, Liu J-F et al (2012) Analysis of alkane-dependent methanogenic community derived from production water of a high-temperature petroleum reservoir. Appl Microbiol Biotechnol 96:1–12

McKay LJ, MacGregor BJ, Biddle JF, Mendlovitz HP, Hoer D, Lipp JS, Lloyd KG, Teske AP (2012) Spatial heterogeneity and underlying geochemistry of phylogenetically diverse orange and white *Beggiatoa* mats in Guaymas Basin hydrothermal sediments. Deep-Sea Res I 67:21–31

McKay L, Klokman V, Mendlovitz H, LaRowe D, Zabel M, Hoer D, Albert D, de Beer D, Amend J, Teske A (2016) Thermal and geochemical influences on microbial biogeography in the hydrothermal sediments of Guaymas Basin. Environ Microbiol Rep 8:150–161

McKay L, Hatzenpichler R, Inskeep WP, Fields MW (2017) Occurrence and expression of novel methyl-coenzyme M reductase gene (*mcrA*) variants in hot spring sediments. Sci Rep 7:7252. https://doi.org/10.1038/s41598-017-07354-x

Mills HJ, Hodges C, Wilson K, MacDonald IR, Sobecky PA (2003) Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. FEMS Microbiol Ecol 46:39–52

Mills HJ, Martinez RJ, Story S, Sobecky PA (2004) Identification of members of the metabolically active microbial populations associated with *Beggiatoa* species mat communities from Gulf of Mexico cold-seep sediments. Appl Environ Microbiol 70:5447–5458

Mills HJ, Martinez RJ, Story S, Sobecky PA (2005) Characterization of microbial community structure in Gulf of Mexico gas hydrates: comparative analysis of DNA- and RNA-derived clone libraries. Appl Environ Microbiol 71:3235–3247

Muyzer G, van der Kraan G (2008) Bacteria from hydrocarbon seep areas growing on short-chain alkanes. Trends Microbiol 16:138–141

Nelson DC, Wirsen CO, Jannasch HW (1989) Characterization of large autotrophic *Beggiatoa* abundant at hydrothermal vents of the Guaymas Basin. Appl Environ Microbiol 55:2909–2917

Niemann H, Lösekann T, De Beer D, Elvert M, Nadalig T, Knittel K, Amann R, Sauter EJ, Schlüter M, Klages M, Foucher JP, Boetius A (2006) Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. Nature 443:854–858

Nobu MK, Narihiro T, Kuroda K, Mei R, Liu W-T (2016) Chasing the elusive Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. ISME J 10:2478–2487

Ollivier B, Cayol JL, Patel BK, Magot M, Fardeau ML, Garcia JL (1997) *Methanoplanus petrolearius* sp. nov., a novel methanogenic bacterium from an oil-producing well. FEMS Microbiol Lett 147:51–56

Ommedal H, Torsvik T (2007) Desulfotignum toluenicum sp. nov., a novel toluene-degrading, sulphate-reducing bacterium isolated from an oil-reservoir model column. Int J Syst Evol Microbiol 57:2865–2869

Orcutt B, Boetius A, Elvert M, Samarkin V, Joye SB (2005) Molecular biogeochemistry of sulfate reduction, methanogenesis, and the anaerobic oxidation of methane at Gulf of Mexico cold seeps. Geochim Cosmochim Acta 69:4267–4281

Orcutt B, Joye SB, Kleindienst S, Knittel K, Ramette A, Rietz A, Samarkin V, Treude T, Boetius A (2010) Impact of natural oil and higher hydrocarbons on microbial diversity, distribution, and activity in Gulf of Mexico cold-seep sediments. Deep-Sea Res II 57:2008–2021

Orphan VJ, Taylor LT, Hafenbradl D, DeLong EF (2000) Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum deposits. Appl Environ Microbiol 66:7000–7111

Orphan VJ, Hinrichs K-U, Ussler W III, Paull CK, Taylor LT, Sylva SP, Hayes JM, DeLong EF (2001a) Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Appl Environ Microbiol 67:1922–1934

Orphan VJ, House CH, Hinrichs K-H, McKeegan KD, DeLong EF (2001b) Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science 293:484–487

Orphan VJ, House CH, Hinrichs K-H, McKeegan KD, DeLong EF (2002) Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proc Natl Acad Sci U S A 99:7663–7668

Parkes JR (1999) Cracking anaerobic bacteria. Nature 401:217–218

Parkes JR, Cragg BA, Wellsbury P (2000) Recent studies on bacterial populations and processes in subseafloor sediments: a review. Hydrogeol J 8:11–28

Pearson A, Seewald JS, Eglinton TI (2005) Bacterial incorporation of relict carbon in the hydrothermal environment of Guaymas Basin. Geochim Cosmochim Acta 69:5477–5486

Pernthaler A, Dekas AE, Brown CT, Goffredi SK, Embaye T, Orphan VJ (2008) Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. Proc Natl Acad Sci U S A 105:7052–7057

Peter JM, Peltonen P, Scott SD, Simoneit BRT, Kawka OE (1991) ^{14}C ages of hydrothermal petroleum and carbonate in Guaymas Basin, Gulf of California: implications for oil generation, expulsion, and migration. Geology 19:253–256

Phelps CD, Kerkhof LJ, Young LY (1998) Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. FEMS Microbiol Ecol 27:269–279

Rabus RR, Nordhaus WL, Widdel F (1993) Complete oxidation of toluene under strictly anoxic conditions by a new sulfate-reducing bacterium. Appl Environ Microbiol 59:1444–1451

Rabus R, Fukui M, Wilkes H, Widdel F (1996) Degradative capacities and 16S rRNA-targeted whole-cell hybridization of sulfate-reducing bacteria in an anaerobic enrichment culture utilizing alkylbenzenes from crude oil. Appl Environ Microbiol 62:3605–3613

Ravot G, Magot M, Fardeau ML, Patel BKC, Prensier G, Egan A, Garcia JL, Ollivier B (1995) *Thermotoga elfii* sp. nov., a novel thermophilic bacterium from an African oil-producing well. Int J Syst Bacteriol 45:308–314

Rees GN, Grassia GS, Sheehy AJ, Dwivedi PP, Patel BKC (1995) *Desulfacinum infernum* gen. nov., sp. nov., a thermophilic sulfate-reducing bacterium from a petroleum reservoir. Int J Syst Bacteriol 45:85–89

Roberts HH, Aharon P, Carney R, Larkin J, Sassen R (1990) Sea floor responses to hydrocarbon seeps, Louisiana continental slope. Geo-Mar Lett 10:232–243

Roberts HH, McBride RA, Coleman J (1999) Outer shelf and slope geology of the Gulf of Mexico: an overview. In: Kumpf H, Steidinger K, Sherman K (eds) The Gulf of Mexico large marine ecosystems: assessment, sustainability, and management. Blackwell Science, Malden, Massachusettts, pp 93–112

Roberts HH, Feng D, Joye SB (2010a) Cold-seep carbonates of the middle and lower continental slope, northern Gulf of Mexico. Deep-Sea Res II 57:2040–2054

Roberts HH, Shedd W, Hunt J (2010b) Dive site geology: DSV ALVIN (2006) and ROV JASON II (2007) dives to the middle-lower continental slope, northern Gulf of Mexico. Deep-Sea Res II Top Stud Oceanogr 57:1837–1858

Roussel EG, Cambon Bonavita M-A, Querellou J, Cragg BA, Webster G, Prieur D, Parkes RJ (2008) Extending the sub-seafloor biosphere. Science 320:1046

Rozanova EP, Pivovarova TA (1988) Reclassification of *Desulfovibrio thermophilus* (Rozanova, Khudyakova, 1974). Microbiol (Engl Tr) 57:102–106

Rubin-Blum M, Antony CP, Borowski C, Sayavedra L, Pape T, Sahling H, Bohrmann G, Kleiner M, Redmond MC, Valentine DL, Dubilier N (2017) Short-chain alkanes fuel mussel and sponge

Cycloclasticus symbionts from deep-sea gas and oil seeps. Nat Microbiol 2:17093. https://doi.org/10.1038/nmicrobiol.2017.93

Rullkötter J, von der Dick H, Welte DH (1982) Organic petrography and extractable hydrocarbons of sediment from the Gulf of California, deep sea drilling project leg 64. In: Curray JR, Blakeslee J, Platt LW, Stout LN, Moore DG, Aguayo JE et al (eds) Initial reports of the deep sea drilling project, vol 64. U.S. Government Printing Office, Washington, DC, pp 837–853

Rüter P, Rabus R, Wilkes H, Aeckersberg F, Rainey FA, Jannasch HW, Widdel F (1994) Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. Nature 372:455–458

Sassen R, Joye S, Sweet ST, DeFreitas DA, Milkov AV, MacDonald IR (1999) Thermogenic gas hydrates and hydrocarbon gases in complex chemosynthetic communities Gulf of Mexico continental slope. Org Geochem 30:485–497

Saunders A, Fornari DJ, Joron JL, Tarney J, Treuil M (1982) Geochemistry of basic igneous rocks, Gulf of California. In: Curray J, Moore D (eds) Initial reports of the deep sea drilling project, vol 64. Ocean Drilling Program, College Station, pp 595–642

Schrader H (1982) Diatom biostratigraphy and laminated diatomaceous sediments from the Gulf of California, deep sea drilling project leg 64. In: Initial reports of the deep sea drilling project, vol 64. U.S. Government Printing Office, Washington, DC, pp 973–981

Schreiber L, Holler T, Knittel K, Meyerdierks A, Amann R (2010) Identification of the dominant sulfate-reducing bacterial partner of anaerobic methanotrophs of the ANME-2 clade. Environ Microbiol 12:2327–2340

Seewald JS, Setfried WE Jr, Shanks WC III. (1994) Variations in the chemical and stable isotope composition of carbon and sulfur species during organic-rich sediment alteration: an experimental and theoretical study of hydrothermal activity at Guaymas Basin, Gulf of California. Geochim Cosmochim Acta 58:5065–5082

Siddique T, Fedorak PM, MacKinnon MD, Foght JM (2007) Metabolism of BTEX and naphtha compounds to methane in oil sands tailings. Environ Sci Technol 41:2350–2356

Siddique T, Penner T, Semple K, Foght JM (2011) Anaerobic biodegradation of longer-chain n-alkanes coupled to methane production in oil sands tailings. Environ Sci Technol 45:5892–5899

Siddique T, Penner T, Klassen J, Nesbø C, Foght JM (2012) Microbial communities involved in methane production from hydrocarbons in oil sands tailings. Environ Sci Technol 46:9802–9810

Simoneit BRT, Bode GR (1982) Appendix II: carbon/carbonate and nitrogen analysis, Leg 64, Gulf of California. In: Curray JR, Blakeslee J, Platt LW, Stout LN, Moore DG, Aguayo JE et al (eds) Initial reports of the deep sea drilling project, vol 64. U.S. Government Printing Office, Washington, DC, pp 1303–1305

So CM, Young LY (1999) Isolation and characterization of a sulfate-reducing bacterium that anaerobically degrades alkanes. Appl Environ Microbiol 65:2969–2976

Stetter KO et al (1993) Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. Nature 365:743–745

Suzuki D, Li Z, Cui X, Zhang C, Katayama A (2014) Reclassification of *Desulfobacterium anilini* as *Desulfatiglans anilini* comb. nov. within *Desulfatiglans* gen. nov., and description of a 4-chlorophenol-degrading sulfate-reducing bacterium, *Desulfatiglans parachlorophenolica* sp. nov. Int J Syst Evol Microbiol 64:3081–3086

Tan B, Nesbø C, Foght J (2014) Re-analysis of omics data indicates *Smithella* may degrade alkanes by addition to fumarate under methanogenic conditions. ISME J 8:2353–2356

Tardy-Jacquenod C, Caumette P, Matheron R, Lanau C, Arnauld O, Magot M (1996) Characterization of sulfate-reducing bacteria isolated from oil-field waters. Can J Microbiol 42:259–266

Teske A, Hinrichs K-U, Edgcomb V, de Vera Gomez A, Kysela D, Sylva SP, Sogin ML, Jannasch HW (2002) Microbial diversity in hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. Appl Environ Microbiol 68:1994–2007

Teske A, Dhillon A, Sogin ML (2003) Genomic markers of ancient anaerobic microbial pathways: sulfate reduction, methanogenesis, and methane oxidation. Biol Bull 204:186–191

Teske A, de Beer D, McKay L, Tivey MK, Biddle JF, Hoer D, Lloyd KG, Lever MA, Røy H, Albert DB, Mendlovitz H, MacGregor BJ (2016) The Guaymas Basin hiking guide to hydrothermal mounds, chimneys and microbial mats: complex seafloor expressions of subsurface hydrothermal circulation. Front Microbiol 7:75. https://doi.org/10.3389/fmicb.2016.00075

Vanwonterghem I, Evans PN, Parks DH, Jensen PD, Woodcroft BJ, Hugenholtz P, Tyson GW (2016) Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. Nat Microbiol 1:1–9. https://doi.org/10.1038/nmicrobiol.2016.170

Voordouw G, Armstrong SM, Reimer MF, Fouts B, Telang AJ, Shen Y, Gevertz D (1996) Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. Appl Environ Microbiol 62:1623–1629

Watanabe K, Watanabe K, Kodama Y, Syutsubo K, Harayama S (2000) Molecular characterization of bacterial populations in petroleum-contaminated groundwater discharged from underground crude oil storage cavities. Appl Environ Microbiol 66:4803–4809

Watanabe K, Kodama Y, Hamamura N, Kaku N (2002) Diversity, abundance, and activity of archaeal populations in oil-contaminated groundwater accumulated at the bottom of an underground crude oil storage cavity. Appl Environ Microbiol 68:3899–3907

Watanabe M, Higashioka Y, Kojima H, Fukui M (2017) *Desulfosarcina widdelii* sp. nov. and *Desulfosarcina alkanivorans* sp. nov., hydrocarbon-degrading sulfate-reducing bacteria isolated from marine sediment, and emended description of the genus *Desulfosarcina*. Int J Syst Evol Microbiol 67:2994–2997

Wawrik B, Marks CR, Davidova IA, McInerney MJ, Pruitt S, Duncan KE, Suflita JM, Callaghan AV (2016) Methanogenic paraffin degradation proceeds via the alkane addition to fumarate by *Smithella* spp. mediated by a syntrophic coupling with hydrogenotrophic methanogens. Environ Microbiol 18:2604–2619

Weber A, Jørgensen BB (2002) Bacterial sulfate reduction in hydrothermal sediments of the Guaymas Basin, Gulf of California, Mexico. Deep-Sea Res I 149:827–841

Wegener G, Krukenberg V, Riedel D, Tegetmeyer HE, Boetius A (2015) Intracellular wiring enables electron transfer between methanotrophic archaea and bacteria. Nature 526:587–590

Welhan JA (1988) Origins of methane in hydrothermal systems. Chem Geol 71:183–1988

Wellsbury P, Goodman K, Barth T, Cragg BA, Barnes SP, Parkes RJ (1997) Deep bacterial biosphere fuelled by increasing organic matter availability during burial and reheating. Nature 388:573–576

Whelan JK, Simoneit BRT, Tarafa ME (1988) C_1–C_8 hydrocarbons in sediments from Guaymas Basin, Gulf of California – comparison to Peru Margin, Japan Trench, and California Borderlands. Org Geochem 12:171–194

Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) The Prokaryotes, 2nd edn. Springer, New York, pp 3352–3378

Widdel F, Rabus R (2001) Anaerobic biodegradation of saturated and aromatic hydrocarbons. Curr Opin Biotechnol 12:259–276

Yang T, Speare K, McKay L, MacGregor BJ, Joye SB, Teske A (2016) Distinct bacterial communities in surficial seafloor sediments following the 2010 Deepwater Horizon blowout. Front Microbiol 7:1384. https://doi.org/10.3389/fmicb.2016.01384

Zengler K, Richnow HH, Rossello-Mora R, Michaelis W, Widdel F (1999) Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 401:266–269

Anaerobic Methane Oxidizers

7

K. Knittel, Gunter Wegener, and A. Boetius

Contents

K. Knittel (✉)
Max Planck Institute for Marine Microbiology, Bremen, Germany
e-mail: kknittel@mpi-bremen.de

G. Wegener
Max Planck Institute for Marine Microbiology, Bremen, Germany

MARUM, Center for Marine Environmental Sciences, Bremen, Germany
e-mail: gwegener@mpi-bremen.de

A. Boetius (✉)
Max Planck Institute for Marine Microbiology, Bremen, Germany

MARUM, Center for Marine Environmental Sciences, Bremen, Germany

Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany
e-mail: antje.boetius@awi.de

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_7

Abstract

The anaerobic oxidation of methane (AOM) with sulfate as the final electron acceptor according to the net reaction $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$ is the major sink of methane in the ocean floor and hence a significant process in the marine methane budget and the global carbon cycle. Since its discovery, much has been learned about the distribution of the AOM process, its activity in different settings, and connections to other metabolic reactions in the seafloor. AOM is performed by consortia of anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB). Since all known ANME and most of their partner bacteria have so far resisted isolation, the physiology of both organisms has been largely inferred from culture-independent approaches on natural enrichments or enrichment cultures. All known ANME are related to methanogenic Euryarchaeota, and as such they reverse the methanogenesis pathway to activate and completely oxidize methane. The reducing equivalents are shuttled to the partner bacteria, which use them for sulfate reduction. Recently, evidence has been found for ANME that can use nitrate or iron as electron acceptors. The exact mechanisms for the required exchange of reducing equivalents in AOM and their genetic codes are yet poorly understood, but recently discovered accumulations of cytochromes and nanowire connections in the intercellular space of the consortia suggest direct electron transfer between both partners.

1 Introduction

First evidence for the removal of methane within anoxic sediments and seawaters came from geochemical observations showing that methane diffuses upward from deeper sediment horizons and disappears in the same zone as sulfate, before any contact with oxygen (Barnes and Goldberg 1976; Martens and Berner 1974; Reeburgh 1982). Global estimates published in the following years suggest that the anaerobic oxidation of methane (AOM) is responsible for the vast majority of methane consumption (~90%) in the seabed; hence, this process has a key role in the marine carbon cycle (Hinrichs and Boetius 2002; Reeburgh 2007). By radiotracer incubations, Zehnder and Brock (1979) investigated the potential of methanogens for methane oxidation and found evidence for the general reversibility of the enzymatic chain of methanogenesis by the detection of ^{14}C transfer from methane into the inorganic carbon pool. These experiments showed that methane is not inert under anoxic conditions. The authors proposed the involvement of archaea, forming a syntrophic partnership with sulfate reducers. Indeed, radioactive tracer incubations with ^{14}C-labeled methane and ^{35}S-labeled sulfate confirmed that methane oxidation coincided with increased sulfate reduction (Iversen and Jørgensen 1985). Field observations and experiments led to the hypothesis of a coupled mechanism in which both methanogenic archaea and sulfate-reducing bacteria (SRB) could profit from AOM, despite the generally low thermodynamic energy yield from this reaction (Hoehler et al. 1994). But many microbiologists were in doubt that the low energy yielded by this reaction could support microbial growth: the free

energy change of AOM under standard conditions at room temperature would be only $\Delta G^\circ = -16$ kJ mol per mol methane oxidized and only slightly higher energy yield at ambient conditions of deep-sea floor, which would have to be shared by the two partners involved in AOM.

First direct evidence for the existence of anaerobic methanotrophic archaea came from Elvert et al. (1999) and Hinrichs et al. (1999), who extracted specific lipid biomarkers from methane-leaking continental slope sediments, the so-called cold seeps of Hydrate Ridge (Cascadia margin off Oregon) and Eel River Basin (off California). The retrieved abundant archaeal lipids such as C_{25} isoprenoids, archaeol and hydroxyarchaeol from these and other sites (Pancost et al. 2000) were conspicuously depleted in the carbon isotope ^{13}C ($\delta^{13}C < -100‰$ vs. Vienna Pee Dee Belemnite), indicating their production from methane-derived carbon. Indeed, the archaeal 16S rRNA gene library from Eel River Basin contained two novel clades of archaeal sequences, which were proposed to derive from anaerobic methane oxidizers (also known as anaerobic methanotrophs, ANME; Hinrichs et al. 1999). Subsequently, the biomarker approach was extended to specifically ^{13}C-depleted distinct bacterial lipids, including $C_{16:1\omega5}$, cyclo$C_{17:0\omega5,6}$, and $C_{17:1\omega6}$ (Boetius et al. 2000; Elvert et al. 2003). Today, intact polar lipids (IPLs) of higher taxonomic specificity such as alkyl glycerol ethers with either glycosidic or phosphate-based headgroups (Biddle et al. 2006; Yoshinaga et al. 2015) can be assigned to different ANME groups.

Further proof for the involvement of syntrophic microbial aggregates in AOM came from their microscopic detection in methane-rich sediments overlaying gas hydrates at Hydrate Ridge (Boetius et al. 2000). Using in situ hybridization with fluorochrome-labeled specific oligonucleotide probes (FISH), these aggregates were identified as dual-species consortia of ANME archaea and partner bacteria. The consortia were shown to be highly abundant representing >90% of the total microbial community in many methane-rich sediments such as found at Hydrate Ridge, in the Black Sea, and the Eel River Basin (Boetius et al. 2000; Orphan et al. 2001; Michaelis et al. 2002). The direct evidence of anaerobic methanotrophy was provided by ion microprobe mass spectrometry, confirming the extreme ^{13}C depletion of the consortia biomass as predicted from the biomarker extractions (Orphan et al. 2002). Meanwhile, the biogeochemical relevance of sulfate-driven AOM and global distribution of different marine ANME clades and consortia types has been confirmed (Holler et al. 2011; Knittel et al. 2005; Ruff et al. 2015). As a third line of evidence for the hypothesis that AOM is performed in a syntrophic association between archaea and bacteria (Zehnder and Brock 1979; Hoehler et al. 1994), in vitro experiments utilizing sediments naturally enriched in ANME demonstrated the stoichiometry of AOM coupled to sulfate reduction (Nauhaus et al. 2002; Holler et al. 2011; Wegener et al. 2016). Culture-independent approaches using metagenomics and -transcriptomics allowed deeper insights into the metabolism of AOM consortia, including the resolution of AOM as reverse methanogenesis (Hallam et al. 2004; Meyerdierks et al. 2010; Wang et al. 2014). The coupling of methane oxidation to sulfate reduction has been explained by direct interspecies electron transfer between the ANME archaea and their partner bacteria, mediated by extracellular cytochromes and nanowire-like structures (McGlynn et al. 2015;

Wegener et al. 2015). Recent studies suggest that specific ANME clades can directly couple methane oxidation to the reduction of alternative electron acceptors. These Experimental evidence support the idea tat ANME can transfer electrons directly to other electron acceptors such as nitrate (Haroon et al. 2013) and iron (Sivan et al. 2014; Ettwig et al. 2016; Weber et al. 2017). Scheller et al (2016) found coupling of methane oxidation to the reduction of the AQDS (9,10-anthraquinone-2,6 disulfonate), an analogue of natural humic acids. Similar findings were reported by Reed et al. (2017) and Valenzuela et al. (2017). The mechanistic details of this direct interspecies electron transfer remain to be enumerated (Walker et al. 2017). Besides ANME archaea, there is one group of freshwater bacteria known to be capable of anaerobic methane oxidation: Ca. Methylomirabilis/clade NC 10 grows in river and lake sediment layers that are methane rich but oxygen poor. These bacteria produce and respire their own oxygen using a nitric oxide dismutase which combines two molecules nitrogen oxide-forming nitrogen and oxygen (Ettwig et al. 2010).

2 Phylogenetic Marker Genes of Anaerobic Methanotrophs

2.1 The 16S rRNA Gene-Based Phylogeny of ANME

In the marine environment, AOM coupled to sulfate reduction is mediated by three distinct clusters of *Euryarchaeota*, namely, ANME-1, ANME-2, and ANME-3. The clusters are related to the orders *Methanosarcinales* and *Methanomicrobiales*, which comprise a major part of the cultivated methanogens (Fig. 1a). Based on their 16S rRNA gene phylogeny, ANME groups are not monophyletic. The phylogenetic distance between the three groups is large with 75–92% sequence similarity, and intergroup similarity of ANME-2 subgroups -2a, -2b, and -2c is comparably low. Thus, members of ANME-1, ANME-2, and ANME-3 certainly belong to different orders or families, which all have the capability to mediate AOM in a wide range of environmental settings. A novel clade related to ANME-2 has been described as the fourth ANME-2 subgroup, ANME-2d (Martinez et al. 2006; Mills et al. 2005). This clade is also known as AOM-associated group (AAA; Knittel and Boetius 2009), denitrifying anaerobic methane oxidation (DAMO, Ettwig et al. 2010), or, more specifically, *Candidatus* Methanoperedens nitroreducens (Haroon et al. 2013). *Candidatus* Methanoperedens nitroreducens has been shown to couple methane oxidation to the reduction of nitrate, a process that might have high relevance particular in eutrophic freshwater environments (Raghoebarsing et al. 2006; Haroon et al. 2013). Furthermore, recent bioreactors, enrichment experiments, and stable-isotope probing suggest members of ANME-2d to couple methane oxidation to iron reduction (Ettwig et al. 2016; Weber et al. 2017) in nitrate-depleted and low-sulfate freshwater systems. Although most of these studies were performed in lakes and wastewater treatment plants, 16S rRNA gene sequences of *Ca*. Methanoperedens/ANME-2d were also retrieved from deep-sea cold seep sulfide chimneys (Schrenk et al. 2003; Reed et al. 2009). Additionally there is evidence for the coupling of AOM to reduction of iron, manganese, and AQDS by ANME-2a and –2c (Beal et al.

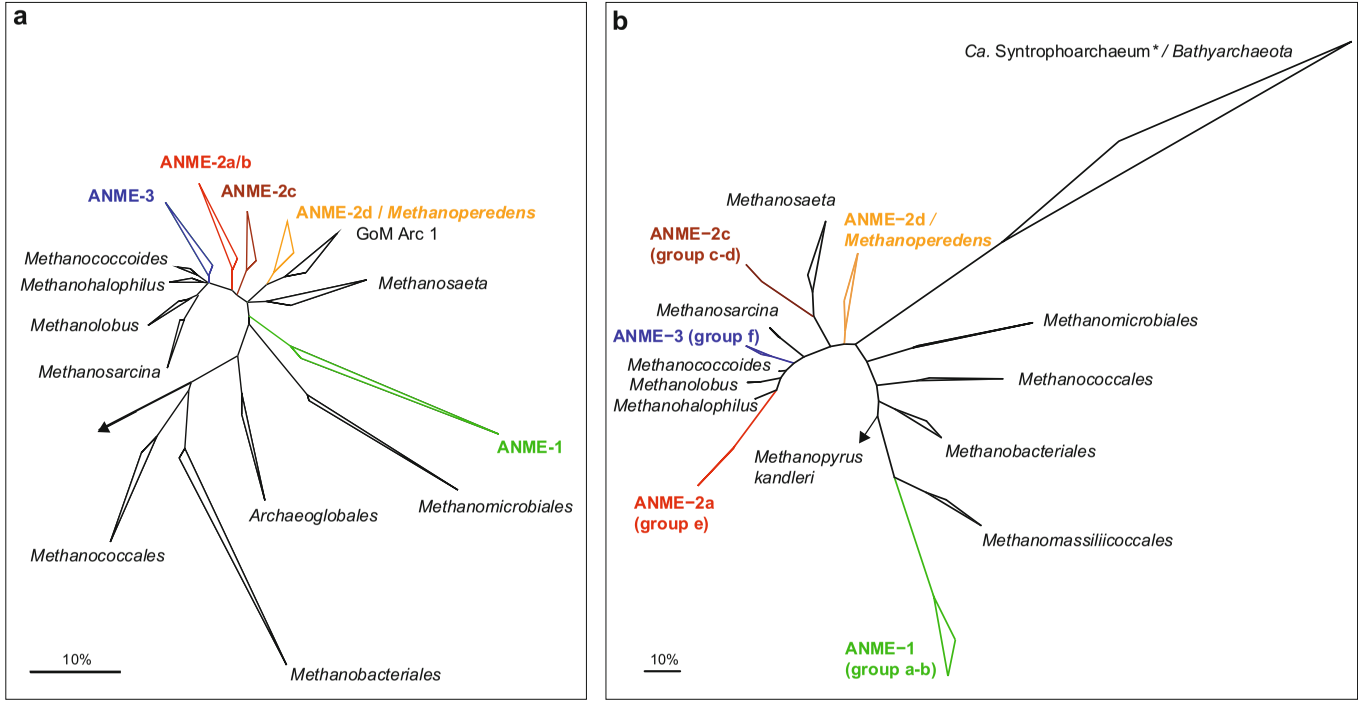

Fig. 1 Phylogenetic trees showing the affiliation of ANME groups to selected reference sequences of the domain *Archaea* based on (**a**) 16S rRNA genes and (**b**) the amino acid sequence of the alpha subunit of methyl CoM reductase (McrA)

2009; Scheller et al. 2016). This suggests that the coupling of AOM to electron acceptors other than sulfate is also possible in marine settings.

2.2 The Methyl-Coenzyme M Reductase (McrA)-Based Phylogeny of ANME

The gene for the alpha subunit of methyl CoM reductase (*mcrA*) encodes the functional part of the Mcr protein. It is used as the key marker for studying the diversity of methanotrophic and methanogenic archaea. Investigations have revealed a remarkably high phylogenetic diversity within McrA among ANME archaea (Knittel and Boetius 2009), which group into four subclusters (Hallam et al. 2003; Lösekann et al. 2007): group a–b (ANME-1), group c–d (ANME-2c), group e (ANME-2a), and group f (ANME-3; Fig. 1b). McrA subunits of ANME-2d are distantly but most closely related (60–72% amino acid identity) to those of ANME-2c. These McrA groups are all distinct from those formed by methanogens. The McrA-based phylogeny of ANME appears to be in parts phylogenetically congruent to the 16S rRNA gene (Fig. 1a, b and Knittel and Boetius 2009). Quantification of ANME-specific *mcrA* genes emerged as an alternative method to 16S rRNA-based fluorescence in situ (FISH) to study distributional patterns of anaerobic methanotrophs in AOM zones (Nunoura et al. 2008). Recently, the role and functioning of Mcr within *Bathyarchaeota* (Evans et al. 2015) and in a specific group of *Methanomicrobia* received rising interest. For instance, *Ca*. Syntrophoarchaeum (formerly referred to as "GoMArch87") apparently combines elements of the methanogenesis pathway with other enzymes to thrive on short-chain hydrocarbons such as propane and butane (Laso-Pérez et al. 2016). To accommodate these larger substrates, their alkane-activating Mcr enzymes are strongly modified as shown by the highly divergent position of their alpha subunits in phylogenetic trees (Fig. 1b).

2.3 The 16S rRNA Gene-Based Phylogeny of ANME Partner Bacteria

ANME archaea form microbial consortia with different sulfate-reducing partner bacteria (Pernthaler et al. 2008; Knittel and Boetius 2009). Most known partner bacteria are SRB belonging to *Deltaproteobacteria*, i.e., SEEP-SRB1a and the Seep-SRB2 branch of *Desulfosarcina*/*Desulfococcus* (DSS; Schreiber et al. 2010) or bacteria affiliated with *Desulfobulbus* (Niemann et al. 2006; Lösekann et al. 2007). These low-temperature-adapted partner bacteria neither grow on external electron donors nor contain gene encoding pathways for independent growth indicating that these partner bacteria are likely obligate syntrophs (Skennerton et al. 2017). In contrast, *Candidatus* Desulfofervidus auxilii (formerly referred to as "HotSeep-1") that was described as partner bacterium of ANME in hydrothermal vent sediments and thermophilic enrichment cultures (Holler et al. 2011; Krukenberg et al. 2016) can be separated from its archaeal partner by providing hydrogen as alternative

energy source (Wegener et al. 2015). Based on the phylogeny of many bacterial marker genes, this bacterium was proposed as the new phylum *Desulfofervidaeota*.

3 Structure and Composition of AOM Consortia

In all marine ecosystems characterized by high methane oxidation rates, ANME organisms have been found in close association with SRB cells, forming microbial consortia of various shapes and composition (Pernthaler et al. 2008; Dekas et al. 2009). ANME-1 cells are typically barrel shaped and may occur as single cells or in short chains of cells. They have also been observed in extremely long multicellular chains exceeding 100 μm in length often with attached partner bacteria (Reitner et al. 2005; Holler et al. 2011). In the Black Sea, "mat-type" associations of ANME-1 archaea and SEEP-SRB1a (Fig. 2a) have been reported (Knittel et al. 2005). Thermophilic ANME-1, however, prefer *Ca*. Desulfofervidus as partner bacterium (Krukenberg et al. 2016; Wegener et al. 2016; Fig. 2b). In laboratory enrichments, the ANME-1 filaments can also coil up and form impressive consortia with SEEP-SRB2 (Wegener et al. 2016). ANME-2a/SEEP-SRB1a consortia often represent the "mixed type," and are not always spherical (Fig. 2c). Typically, ANME-2c/SEEP-SRB1a consortia represent the well-known "shell-type" with an inner core of ANME-2, which is partially or fully surrounded by an outer shell of SRB (Fig. 2d; Knittel et al. 2005). These SRB are physically attached to ANME-2 archaea, forming cell aggregates covered by a thick organic matrix. The morphology of the SEEP-SRB1a cells varies from cocci (mostly associated with ANME-2c cells) to rod shaped or vibrioform (often associated with ANME-2a cells) suggesting that they might belong to different species. ANME-2a and ANME-2c consortia can also be found in association with SEEP-SRB2 as partner bacteria (Kleindienst et al. 2012; Wegener et al. 2016).

ANME-3 archaea also form shell-type consortia with *Desulfobulbus*-related bacteria as sulfate-reducing partner (Fig. 2e; Lösekann et al. 2007; Niemann et al. 2006); however, only very few bacteria are associated. Shell-type consortia have been observed to grow by an increase in the number as well as in the diameter of the aggregates (Nauhaus et al. 2007). Starting from few archaea and SRB cells, the consortia seem to develop from small to big aggregations of up to 100,000 cells (Nauhaus et al. 2007). Larger consortia divide evenly into two or unevenly into more aggregates when SRB grow into the archaeal core. In situ, the average diameter of consortia is 3–5 μm with the largest detected consortium of >20 μm. Even much larger consortia have been observed in enrichment cultures with >50 μm in diameter (Nauhaus et al. 2007; Wegener et al. 2016). The AOM consortia enriched in the lab constitute roughly 2/3 by ANME biomass which is supported by about 90% of all transcriptomic reads assigned to ANME (Krukenberg et al. 2018).

All known ANME are autofluorescent under UV light, a feature typical for methanogenic and methanotrophic archaea that is based on the presence of large amounts of cofactor F_{420} as electron carrier (Fig. 2f). Recently, Hatzenpichler et al. (2016) successfully applied bioorthogonal noncanonical amino acid tagging

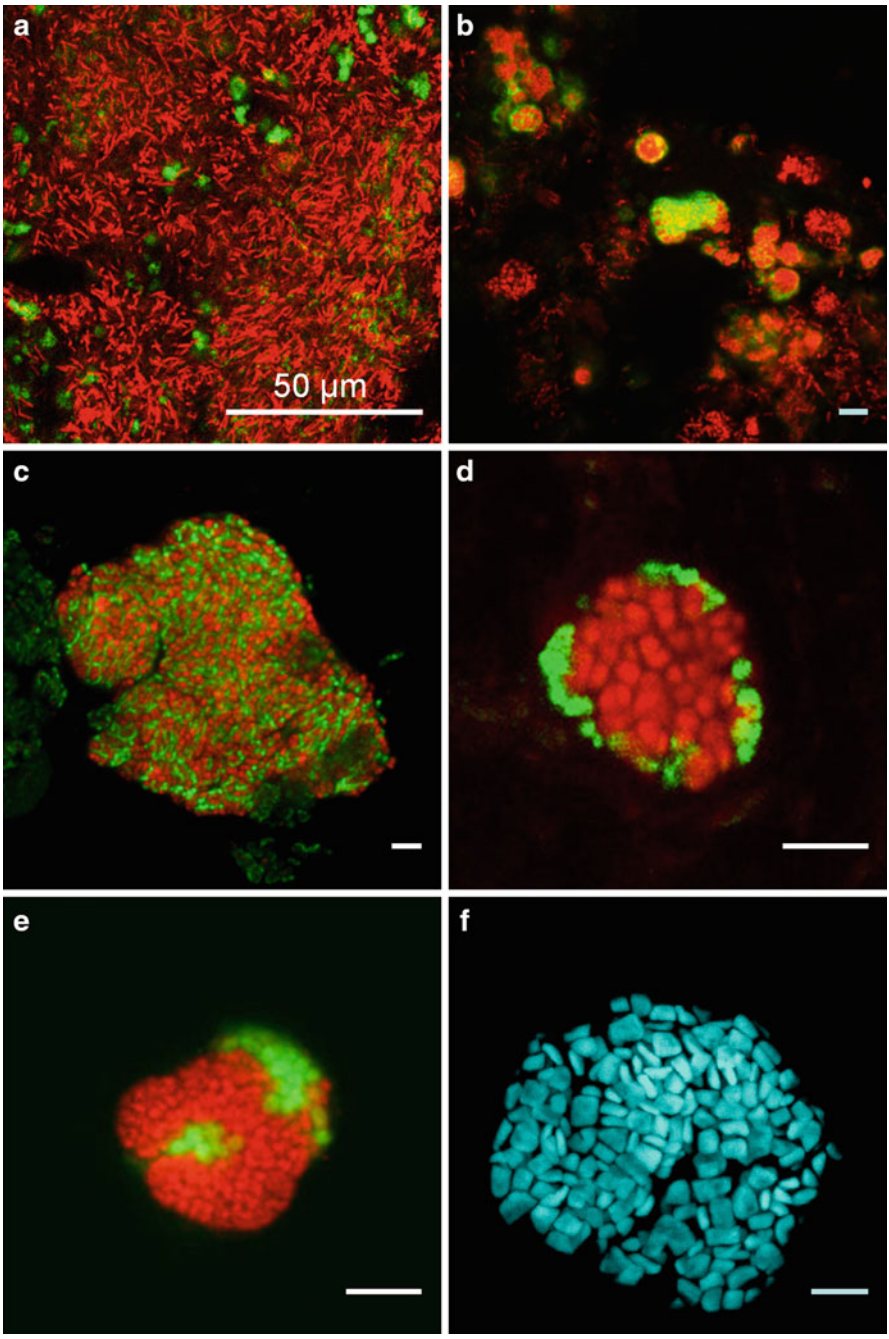

Fig. 2 (continued)

fluorescence in situ hybridization (BONCAT-FISH) on the slow-growing AOM consortia. This allowed a direct visualization of the translational activity of different ANME clades and their specific partner cells. Other helpful approaches to link phylogenetic analysis and translational activity at the level of individual consortia include the combination of MagnetoFISH and/or FISH-NanoSIMS with a parallel transcription analysis of marker genes (Trembath-Reichert et al. 2016; Dekas et al. 2016).

4 Habitats of Anaerobic Oxidizers of Methane

The most prominent habitats of ANME archaea are cold seeps and hot vents (Fig. 3), where ANME make up the majority of the microbial biomass in sediments. Marine cold seeps are geosystems characterized by migration and discharge of hydrocarbons, mostly methane, from the subsurface seabed to the hydrosphere. Seep ecosystems from Eel River Basin (Orphan et al. 2001), Hydrate Ridge (Boetius et al. 2000), Black Sea (Michaelis et al. 2002), Gulf of Mexico (Lloyd et al. 2006; Orcutt et al. 2005), and the Tommeliten and Gullfaks area in the North Sea (Wegener et al. 2008b), as well as mud volcanoes from the Mediterranean Sea (Omoregie et al. 2008) and the Barents Sea (Lösekann et al. 2007), harbor dense ANME populations of $>10^{10}$ cells cm^{-3}. Methane oxidation rates up to several micromol per cubic centimeter and day are measured. With the exception of the unique microbial reef ecosystems in the Black Sea, most of the studies indicate a dominance of ANME-2 in sulfate-penetrated near-surface sediments (top 10 cm) and of ANME-3 in some mud volcano systems (Lösekann et al. 2007). A prominent example is the Hydrate Ridge with hot spots of ANME-2 (up to 10^8 consortia cm^{-3}) just above surface gas hydrate deposits making up more than 90% of microbial biomass at this site. Interestingly, ANME-2 subgroups revealed different preferences for either *Beggiatoa* (ANME-2a) or *Calyptogena* (ANME-2c) fields (Knittel et al. 2005), indicating that different environmental conditions select for different ANME groups.

ANME-1 dominate (1) sediments overlaying a methane-rich brine pool in the Gulf of Mexico (ANME-1b; Lloyd et al. 2006) and (2) microbial mats from Black Sea cold seeps (ANME-1a, -b; Knittel et al. 2005). In the Black Sea, in water depths >180 m, giant reef-like structures composed of porous carbonates and microbial mats growing vertically or horizontally were found on the seafloor

Fig. 2 Epifluorescence micrographs of different ANME consortia visualized by FISH or catalyzed reporter deposition CARD-FISH. (**a**) "Mat-type" consortium formed by ANME-1 (red) and DSS cells (green); (**b**) thermophilic consortia of ANME-1 (red) and *Desulfofervidus* sp. (green) in an enrichment culture; (**c**) "mixed-type" consortium of ANME-2a (red) and DSS (green); (**d**) "shell-type" consortia of ANME-2c (red) and DSS (green) cells; (**e**) ANME-3/*Desulfobulbus* consortium (archaea in red, bacteria in green); (**f**) autofluorescence of ANME consortium under UV light caused by the presence of large amounts of coenzyme F_{420} as electron carrier (courtesy, V. Krukenberg). Unless otherwise indicated, scale bar is 5 μm

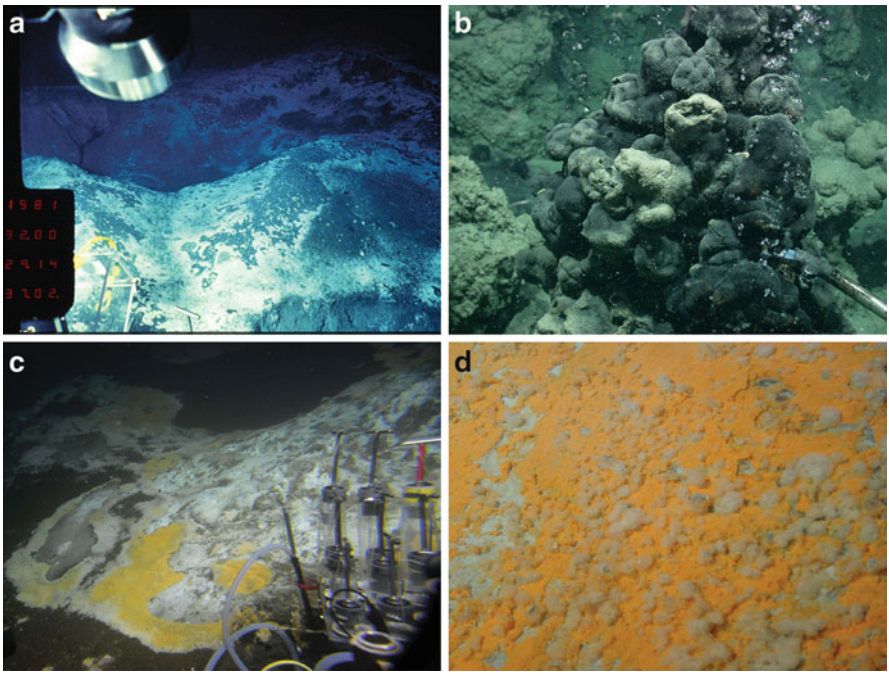

Fig. 3 Different types of hot spot habitats for anaerobic methanotrophy. (**a**) Bacterial mats at the southern summit of Hydrate Ridge off Oregon. (**b**) The Black Sea microbial reefs at 250 m water depth off the Crimean peninsula. (**c**) Sedimentary hydrothermal systems at Guaymas Basin. (**d**) Brine pool influenced methane seeps at Eastern Mediterranean mud volcanoes

(Krüger et al. 2008; Stadnitskaia et al. 2005) These mats have been shown to mediate AOM and consist mainly of densely aggregated ANME-1 cells and different SRB (Knittel et al. 2005; Michaelis et al. 2002; Reitner et al. 2005). Treude et al. (2007) combined radiotracer incubations with CARD-FISH and secondary ion mass spectrometry (SIMS) to locate hot spots of methanotrophy, which were found close to the mat surface associated with dense associations of microcolonies of ANME-1 archaea and SRB of the DSS cluster. Generally, the Black Sea mats are very heterogeneous and also provide niches for ANME-2. Especially the black nodules from the top of the reef seem to be dominated by ANME-2 as shown by specific ^{13}C-depleted lipids and FISH (Blumenberg et al. 2004; Krüger et al. 2008).

High methane fluxes are also found at hydrothermal vents of mid-ocean ridges; nevertheless these ecosystems often harbor only few niches for ANME communities. The seafloor consists of basalts and usually lacks a sediment cover. Moreover the rising fluids of most hydrothermal vents are sulfate free; hence the niches for ANME are limited to small anoxic zones within vent chimneys and rocks or usually rare sediment patches. In contrast, sedimentary hydrothermal systems such as the Guaymas Basin offer more suitable habitats within the surface seafloor (Teske et al. 2002; Schouten et al. 2003; Kallmeyer and Boetius

2004; Holler et al. 2011; Wegener et al. 2015). In sediment samples from this site, high methane oxidation and sulfate reduction rates up to 1 μmol per cubic centimeter and day were measured. ANME-1-dominated methanotrophic cultures could be retrieved from these sediments and sustained at temperatures of up to 60 °C (Holler et al. 2011). Sequences from ANME-1 archaea were also retrieved from extreme habitats such as the Lost City hydrothermal field with a temperature range from <40 °C to 90 °C and a pH of 9–11 (Kelley et al. 2005; Brazelton et al. 2006). Furthermore, sequences of ANME archaea were found in acidic sediment (pH 4) overlying a submarine CO_2 lake (Inagaki et al. 2006) and in barite chimneys (Steen et al. 2016), indicating that AOM might also occur at even harsher temperatures and chemical conditions. Different kinds of ANME habitats are seep carbonates, which are mineral precipitates derived as a by-product of AOM. Recently it was found that even those exposed carbonates, which are deemed paleo-recorders of seepage activity, can harbor active microbial communities, and serve as dynamic methane sinks (Marlow et al. 2014; Case et al. 2015).

In diffusive seabed systems, the distribution of ANME is restricted to sulfate-methane transition zones (SMTZ), because this is the only place where both methane and sulfate are available. The phylogenetic compositions of ANME populations and their sulfate-reducing partner bacteria are comparable to those at cold seeps. Both ANME-1 and ANME-2 archaea occur in SMTZ sediments: Eckernförde Bay sediments (German Baltic, Treude et al. 2005), for example, are clearly dominated by ANME-2 archaea, while ANME-1 are dominant, e.g., in the deep SMTZ of the Tommeliten seep area (Niemann et al. 2005). The ANME population densities in SMTZ are, however, are with $<10^6$ cells cm^{-3} much lower than in cold seeps (Niemann et al. 2005), and associated AOM rates are, depending on the methane and sulfate concentration gradient, in the pico- to low nanomolar range per cubic centimeter and day. Most likely, the SMTZ-ANME archaea represent the seed populations for cold seep communities. It is still unknown whether these ANME possess special physiological adaptations to their energetically less favorable habitat compared to those at cold seeps. A global assessment of the distribution of ANME and sulfate-reducing partners at cold seeps, vents, and SMTZ zones shows that these occur worldwide but are locally selected by the environment (Ruff et al. 2015). Globally, a few cosmopolitan phylotypes show high relative sequence abundances, suggesting high population densities and global relevance for the control of methane emission from the seafloor. These are associated with a substantial diversity of rare relatives, indicating substantial diversification.

Beyond marine habitats, ANME and their sulfate-reducing partners have been detected in terrestrial habitats including various freshwater and polar environments, suggesting that global relevance of the ANME group in carbon cycling is still underestimated. Recent findings include the presence in shallow coastal saline lakes (Bhattarai et al. 2017), sub-Arctic lake sediments (Martinez-Cruz et al. 2017), tropical wetlands (Valenzuela et al. 2017), temperate lake waters and sediments (Weber et al. 2017; Roland et al. 2017), and carbonate in continental crystalline crust (Drake et al. 2017).

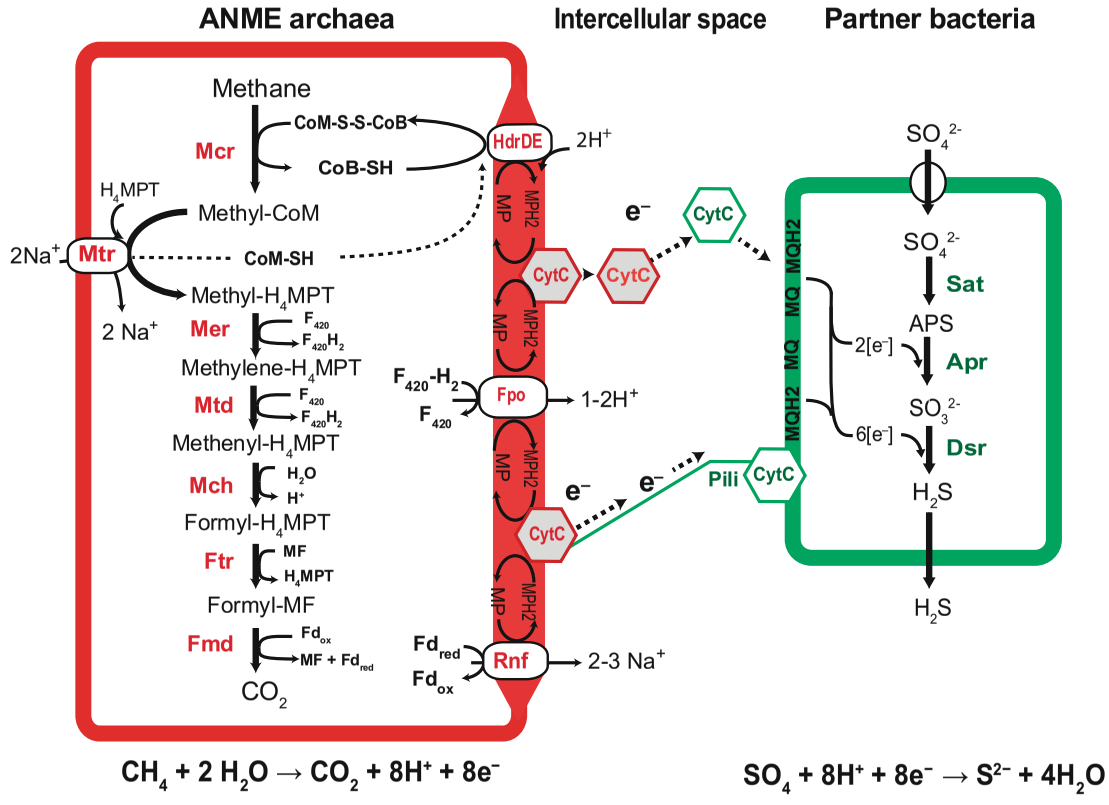

Fig. 4 (continued)

5 The Physiology of ANME Archaea and Their Partner Bacteria

Surveys of metagenomic libraries revealed the presence of nearly all genes typically associated with methanogenesis in ANME and supported the hypothesis that AOM is in principle a reversal of methanogenesis (Hallam et al. 2004; Meyerdierks et al. 2005; Stokke et al. 2012). The physiologically most intriguing question is as to the enzymatic functioning and kinetics of the activation of methane. Krüger et al. (2003) extracted a prominent nickel compound from AOM-mediating microbial mats displaying the same absorption spectrum as the authentic cofactor F_{430} of the Mcr, but with a slightly higher molecular mass (951 Da). Assuming a modified type of the methanogenic Mcr, mediating the terminal step in methanogenesis, the reverse Mcr reaction may strongly limit the rate of the formation of the initial intermediate, methyl-coenzyme M, as discussed elsewhere (Shima and Thauer 2005). Consequently, to provide substantial amounts of this protein, its encoding subunits are upon the highest expressed genes in all studied AOM enrichments (Wegener et al. 2015; Krukenberg et al. 2018). The encoded protein contributes up to 7% of total extracted mat proteins (Shima and Thauer 2005).

The complete oxidation of the produced methyl CoM toward CO_2 proceeds on the upstream part of the methanogenesis pathway (Hallam et al. 2004; Fig. 4). ANME-1 archaea have modified this pathway likely by exchanging the F_{420}-dependent N^5,N^{10}-methylene-tetrahydromethanopterin reductase (Mer) by the NADH-dependent methylenetetrahydrofolate reductase (Met; Stokke et al. 2012; Krukenberg et al. 2018). The metabolic reason for this modification is yet unclear. All known ANME archaea involved in sulfate-dependent AOM do not have a pathway for dissimilatory sulfate reduction and hence depend on the activity of partner bacteria.

Fig. 4 Metabolic scheme of the current understanding of the physiology of AOM with sulfate in ANME-2 and partner bacteria. ANME archaea (red) oxidize methane by reversing all the steps of the methanogenesis pathway. Via cytoplasmic and membrane-based electron carriers such as F_{420} and ferrodoxin, methanophenazine and cytochromes reducing equivalents are moved to and across the cell membrane, from where they are transferred to the partner bacteria (green) which perform sulfate reduction. Recent data suggest interspecies electron transfer via extracellular-type cytochromes or conductive pili (Wegener et al. 2015; McGlynn et al. 2015). *Abbreviations*: ANME, *Mcr* methyl CoM reductase, *Mtr* methyl transferase (membrane bound), *Mer* N^5,N^{10}-methylene-tetrahydromethanopterin reductase, *Mtd* methylenetetrahydromethanopterin dehydrogenase (Mtd), *Mch* F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase, *Ftr* formylmethanofuran-tetrahydromethanopterin formyltransferase, *Fmd* formylmethanofuran dehydrogenase, *HdrDE* membrane-based heterodisulfide reductase, MP(H2) methanophenazine, *fpo* H_2F_{420}:phenazine oxidoreductase, *Rnf* ferredoxin:NAD+ oxidoreductase, *cytC* cytochrome C. Partner bacterium, *MQ* menaquinone, membrane electron carrier found in AOM partner bacteria (Krukenberg et al. 2016), *Sat* adenylyl:sulfate transferase, *Apr* APS reductase, *Dsr* dissimilatory sulfite reductase. For modifications of reverse methanogenesis in ANME-1 archaea, see Stokke et al. (2012); for modifications of electron flow in *Ca*. Methanoperedens, see Haroon et al. (2013) and Arshad et al. (2015)

The consumption of methane and simultaneous formation of sulfide from sulfate at a molar ratio of approximately 1:1 was shown by mass balancing AOM reactants and products in enrichment experiments (Nauhaus et al. 2002; Holler et al. 2011) or radioisotope incubations (Ruff et al. 2016) and is in accordance with the stoichiometric equation of AOM. The stoichiometry of AOM also indicates that methane oxidation is quantitatively coupled to sulfate reduction and that only 1% of the released reducing equivalents are used for carbon fixation of both involved organisms (Nauhaus et al. 2007; Wegener et al. 2008b, 2016). This is in contrast to aerobic methanotrophy, in which up to 60% of the methane carbon is used for biosynthesis (Leak and Dalton 1986). The low biomass yield in AOM is accompanied by a low biomass doubling time ranging from <2 months to approximately 7 months (Nauhaus et al. 2007; Holler et al. 2011). The slow growth may be related to the bioenergetic limitations caused by the minimal energy yield of AOM (Nauhaus et al. 2007). For instance, within sulfate-methane transition zones, active AOM was measured at conditions that allow free energy yields for sulfate-dependent AOM of not more than -10 kJ mol^{-1} (Dale et al. 2008) which should be shared by two organisms.

Based on current knowledge, the association of marine ANME with sulfate-reducing partner bacteria is a syntrophic interaction in which the methanotrophic archaeon activates and fully oxidizes the methane to CO_2 (Hallam et al. 2003). The reducing equivalents are scavenged as electron donor by the sulfate-reducing partner (Hoehler et al. 1994; Nauhaus et al. 2002; Valentine and Reeburgh 2000). So far, all information generated from biogeochemical data including natural and experimental isotope labeling, tracer measurements, enrichment experiments, and metagenomic and proteomic analyses do not falsify this hypothesis. Stable carbon isotope labeling experiments have shown that the growth of methanotrophic archaea and their partner bacteria is coupled and depends on the presence of methane and that the SRB partner appears to grow autotrophically (Wegener et al. 2008a; Kellermann et al. 2012), which excluded the exchange of methane-derived organic intermediates. Furthermore, feeding studies in the absence of methane with conventional methanogenic substrates such as H_2, formate, acetate, or methanol were never observed to lead to the growth of the SRB, except for one thermophilic strain capable of switching to a free-living mode that alternatively feeds on hydrogen (Wegener et al. 2015, 2016). It was also proposed that ANME perform incomplete sulfate reduction to zero-valent sulfur compounds, whereas their partner bacteria would disproportionate these compounds (Milucka et al. 2012). However, investigations with a variety of AOM enrichment cultures did not confirm the previously observed disproportionation reaction (Wegener et al. 2016).

As for syntrophic interaction mode, direct electron transfer was suggested (Thauer and Shima 2008; Summers et al. 2010). This hypothesis is strongly supported by modeling and detection of abundant cytochromes and nanowire-like structures in the intercellular space (Fig. 4; McGlynn et al. 2015; Wegener et al. 2015). Recently, this interaction mechanism was likewise detected in short-chain hydrocarbon-degrading microbial consortia, suggesting a widespread mechanism in archaeal-bacterial consortia (Laso-Pérez et al. 2016).

Recent progress and details on ANME physiology are summarized in McGlynn (2017) and Timmers et al. (2017).

6 Research Needs

The anaerobic oxidation of methane is a key biogeochemical process on Earth and represents a globally important sink for methane. Especially with regard to the feedback mechanisms to global warming, their role in controlling methane fluxes in the seabed and in other aquatic habitats is an important question, especially where gas hydrates and frozen soils move to the physicochemical stability limit. Marine anaerobic methanotrophic archaea are present in all environments where methane and sulfate overlap, but little is known on the environmental selection pressures for the different ANME clades and their partner bacteria. A rising number of published genome drafts might help to generate new hypotheses on the functioning and metabolic differences between the different ANME communities. Several recent enrichment studies, molecular approaches, and electron microscopy indicate a syntrophy via interspecies electron transfer and the capacity of direct electron shuttling to a larger variety of electron acceptors including electrodes. However, the underlying biochemical mechanisms remain unknown. Answering such questions from metagenomic data sets remains demanding due to the high proportion of open reading frames that cannot be assigned to certain functional categories. The ability to grow the bacterial partners independently from ANME uncovering the AOM enigma. In the future, cultivation of ANME in microbial fuel cells might allow growth of these otherwise strictly syntrophic organisms independent from their partners.

References

Arshad A, Speth DR, de Graaf RM, Op den Camp HJM, Jetten MSM, Welte CU (2015) A metagenomics-based metabolic model of nitrate-dependent anaerobic oxidation of methane by Methanoperedens-like archaea. Front Microbiol 6:1423

Barnes RO, Goldberg ED (1976) Methane production and consumption in anoxic marine sediments. Geology 4:297–300

Beal EJ, House CH, Orphan VJ (2009) Manganese- and iron-dependent marine methane oxidation. Science 325:184–187

Bhattarai S, Cassarini C, Gonzalez-Gil G, Egger M, Slomp CP, Zhang Y, Esposito G, Lens PN (2017) Anaerobic methane-oxidizing microbial community in a coastal marine sediment: anaerobic methanotrophy dominated by ANME-3. Microb Ecol 74:608–622

Biddle JF, Lipp JS, Lever MA, Lloyd KG, Sorensen KB, Anderson R, Fredricks HF, Elvert M, Kelly TJ, Schrag DP, Sogin ML, Brenchley JE, Teske A, House CH, Hinrichs K-U (2006) Heterotrophic archaea dominate sedimentary subsurface ecosystems off Peru. Proc Natl Acad Sci U S A 103:3846–3851

Blumenberg M, Seifert R, Reitner J, Pape T, Michaelis W (2004) Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. Proc Natl Acad Sci U S A 101:11111–11116

Boetius A, Ravenschlag K, Schubert C, Rickert D, Widdel F, Gieseke A, Amann R, Jørgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626

Brazelton WJ, Schrenk MO, Kelley DS, Baross JA (2006) Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. Appl Environ Microbiol 72:6257–6270

Case DH, Pasulka AL, Marlow JJ, Grupe BM, Levin LA, Orphan VJ (2015) Methane seep carbonates host distinct, diverse, and dynamic microbial assemblages. MBio 6:e01348–e01315

Dale AW, Regnier P, Knab NJ, Jorgensen BB, Van Cappellen P (2008) Anaerobic oxidation of methane (AOM) in marine sediments from the Skagerrak (Denmark): II. Reaction-transport modeling. Geochim Cosmochim Acta 72:2880–2894

Dekas AE, Poretsky RS, Orphan VJ (2009) Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. Science 326:422–426

Dekas AE, Connon SA, Chadwick GL, Trembath-Reichert E, Orphan VJ (2016) Activity and interactions of methane seep microorganisms assessed by parallel transcription and FISH-NanoSIMS analyses. ISME J 10:678–692

Drake H, Heim C, Roberts NM, Zack T, Tillberg M, Broman C, Ivarsson M, Whitehouse MJ, Åström ME (2017) Isotopic evidence for microbial production and consumption of methane in the upper continental crust throughout the Phanerozoic eon. Earth Planet Sci Lett 470:108–118

Elvert M, Suess E, Whiticar MJ (1999) Anaerobic methane oxidation associated with marine gas hydrates: superlight C-isotopes from saturated and unsaturated C_{20} and C_{25} irregular isoprenoids. Naturwissenschaften 86:295–300

Elvert M, Boetius A, Knittel K, Jørgensen BB (2003) Characterization of specific membrane fatty acids as chemotaxonomic markers for sulfate-reducing bacteria involved in anaerobic oxidation of methane. Geomicrobiol J 20:403–419

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MM et al (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464:543–548

Ettwig KF, Zhu B, Speth D, Keltjens JT, Jetten MS, Kartal B (2016) Archaea catalyze iron-dependent anaerobic oxidation of methane. Proc Natl Acad Sci U S A 113:12792–12796

Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD, Tyson GW (2015) Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. Science 350:434–438

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

Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, Richardson PM, DeLong EF (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. Science 305:1457–1462

Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P, Yuan Z, Tyson GW (2013) Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature 500:567–570

Hatzenpichler R, Connon SA, Goudeau D, Malmstrom RR, Woyke T, Orphan VJ (2016) Visualizing in situ translational activity for identifying and sorting slow-growing archaeal – bacterial consortia. Proc Natl Acad Sci U S A 113:E4069–E4078

Hinrichs K-U, Boetius A (2002) The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. In: Wefer G, Billett D, Hebbeln D, Jørgensen BB, Schlüter M, van Weering T (eds) Ocean margin systems. Springer, Berlin, pp 457–477

Hinrichs KU, Hayes JM, Sylva SP, Brewer PG, DeLong EF (1999) Methane-consuming archaebacteria in marine sediments. Nature 398:802–805

Hoehler TM, Alperin MJ, Albert DB, Martens CS (1994) Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for a methanogen-sulfate reducer consortium. Glob Biogeochem Cycles 8:451–463

Holler T, Widdel F, Knittel K, Amann R, Kellermann MY, Hinrichs K-U, Teske A, Boetius A, Wegener G (2011) Thermophilic anaerobic oxidation of methane by marine microbial consortia. ISME J 5:1946–1956

Inagaki F, Kuypers MMM, Tsunogai U, Ishibashi J, Nakamura K, Treude T, Ohkubo S, Nakaseama M, Gena K, Chiba H, Hirayama H, Nunoura T, Takai K, Jørgensen BB, Horikoshi K, Boetius A (2006) Microbial community in a sediment-hosted CO_2 lake of the southern Okinawa Trough hydrothermal system. Proc Natl Acad Sci U S A 103:14164–14169

Iversen N, Jørgensen JN (1985) Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). Limnol Oceanogr 30:944–955

Kallmeyer J, Boetius A (2004) Effects of temperature and pressure on sulfate reduction and anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. Appl Environ Microbiol 70:1231–1233

Kellermann MY, Wegener G, Elvert M, Yoshinaga MY, Lin Y-S, Holler T, Mollar XP, Knittel K, Hinrichs K-U (2012) Autotrophy as a predominant mode of carbon fixation in anaerobic methane-oxidizing microbial communities. Proc Natl Acad Sci U S A 109:19321–19326

Kelley DS, Karson JA, Fruh-Green GL, Yoerger DR, Shank TM, Butterfield DA, Hayes JM, Schrenk MO, Olson EJ, Proskurowski G, Jakuba M, Bradley A, Larson B, Ludwig K, Glickson D, Buckman K, Bradley AS, Brazelton WJ, Roe K, Elend MJ, Delacour A, Bernasconi SM, Lilley MD, Baross JA, Summons RE, Sylva SP (2005) A serpentinite-hosted ecosystem: the lost City hydrothermal field. Science 307:1428–1434

Kleindienst S, Ramette A, Amann R, Knittel K (2012) Distribution and in situ abundance of sulfate-reducing bacteria in diverse marine hydrocarbon seep sediments. Environ Microbiol 14:2689–2710

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

Knittel K, Lösekann T, Boetius A, Kort R, Amann R (2005) Diversity and distribution of methanotrophic archaea at cold seeps. Appl Environ Microbiol 71:467–479

Krüger M, Meyerdierks A, Glöckner FO, Meyerdierks A, Amann R, Widdel F, Kube M, Reinhardt R, Kahnt J, Bocher R, Thauer RK, Shima S (2003) A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. Nature 426:878–881

Krüger M, Blumenberg M, Kasten S, Wieland A, Kanel L, Klock J-H, Michaelis W, Seifert R (2008) A novel, multi-layered methanotrophic microbial mat system growing on the sediment of the Black Sea. Environ Microbiol 10:1934–1947

Krukenberg V, Harding K, Richter M, Glöckner FO, Gruber-Vodicka HR, Adam B, Berg JS, Knittel K, Tegetmeyer HE, Boetius A, Wegener G (2016) *Candidatus* Desulfofervidus auxilii, a hydrogenotrophic sulfate-reducing bacterium involved in the thermophilic anaerobic oxidation of methane. Environ Microbiol 18:3073–3091

Krukenberg V, Riedel D, Gruber-Vodicka HR, Buttigieg PL, Tegetmeyer HE, Boetius A, Wegener, G (2018) Gene expression and ultrastructure of meso- and thermophilic methanotrophic consortia. Environ Microbiol 20:1651–1666

Laso-Pérez R, Wegener G, Knittel K, Widdel F, Harding KJ, Krukenberg V, Meier DV, Richter M, Tegetmeyer HE, Riedel D, Richnow H-H, Adrian L, Reemtsma T, Lechtenfeld OJ, Musat F (2016) Thermophilic archaea activate butane via alkyl-coenzyme M formation. Nature 539:396–401

Leak DJ, Dalton H (1986) Growth yields of methanotrophs. Appl Microbiol Biotechnol 23:470–476

Lloyd KG, Lapham L, Teske A (2006) An anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. Appl Environ Microbiol 72:7218–7230

Lösekann T, Knittel K, Nadalig T, Fuchs B, Niemann H, Boetius A, Amann R (2007) Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano, Barents Sea. Appl Environ Microbiol 73:3348–3362

Marlow JJ, Steele JA, Ziebis W, Thurber AR, Levin LA, Orphan VJ (2014) Carbonate-hosted methanotrophy represents an unrecognized methane sink in the deep sea. Nat Commun 5:5094

Martens CS, Berner RA (1974) Methane production in the interstitial waters of sulfate-depleted marine sediments. Science 185:1167–1169

Martinez RJ, Mills HJ, Story S, Sobecky PA (2006) Prokaryotic diversity and metabolically active microbial populations in sediments from an active mud volcano in the Gulf of Mexico. Environ Microbiol 8:1783–1796

Martinez-Cruz K, Leewis M-C, Herriott IC, Sepulveda-Jauregui A, Anthony KW, Thalasso F, Leigh MB (2017) Anaerobic oxidation of methane by aerobic methanotrophs in sub-Arctic lake sediments. Sci Total Environ 607:23–31

McGlynn SE (2017) Energy metabolism during anaerobic methane oxidation in ANME archaea. Microbes Environ 32:5–13

McGlynn SE, Chadwick GL, Kempes CP, Orphan VJ (2015) Single cell activity reveals direct electron transfer in methanotrophic consortia. Nature 526:532–535

Meyerdierks A, Kube M, Lombardot T, Knittel K, Bauer M, Glöckner FO, Reinhardt R, Amann R (2005) Insights into the genomes of archaea mediating the anaerobic oxidation of methane. Environ Microbiol 7:1937–1951

Meyerdierks A, Kube M, Kostadinov I, Teeling H, Glockner FO, Reinhardt R, Amann R (2010) Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group. Environ Microbiol 12:422–439

Michaelis W, Seifert R, Nauhaus K, Treude T, Thiel V, Blumenberg M, Knittel K, Gieseke A, Peterknecht K, Pape T, Boetius A, Amann R, Jørgensen BB, Widdel F, Peckmann J, Pimenov NV, Gulin MB (2002) Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science 297:1013–1015

Mills HJ, Martinez RJ, Story S, Sobecky PA (2005) Characterization of microbial community structure in Gulf of Mexico gas hydrates: comparative analysis of DNA- and RNA-derived clone libraries. Appl Environ Microbiol 71:3235–3247

Milucka J, Ferdelman TG, Polerecky L, Franzke D, Wegener G, Schmid M, Lieberwirth I, Wagner M, Widdel F, Kuypers MMM (2012) Zero-valent sulphur is a key intermediate in marine methane oxidation. Nature 491:541–546

Nauhaus K, Boetius A, Krüger M, Widdel F (2002) In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. Environ Microbiol 4:296–305

Nauhaus K, Albrecht M, Elvert M, Boetius A, Widdel F (2007) In vitro cell growth of marine archaeal-bacterial consortia during anaerobic oxidation of methane with sulfate. Environ Microbiol 9:187–196

Niemann H, Elvert M, Hovland M, Orcutt B, Judd A, Suck I, Gutt J, Joye S, Damm E, Finster K, Boetius A (2005) Methane emission and consumption at a North Sea gas seep (Tommeliten area). Biogeosciences 2:335–351

Niemann H, Liemann T, DeBeer D, Elvert M, Nadalig T, Knittel K, Amann R, Sauter EJ, Schlüter M, Klages M, Foucher JP, Boetius A (2006) Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. Nature 443:854–858

Nunoura T, Oida H, Miyazaki J, Miyashita A, Imachi H, Takai K (2008) Quantification of *mcrA* by fluorescent PCR in methanogenic and methanotrophic microbial communities. FEMS Microbiol Ecol 64:240–247

Omoregie EO, Mastalerz V, de Lange G, Straub KL, Kappler A, Roy H, Stadnitskaia A, Foucher J-P, Boetius A (2008) Biogeochemistry and community composition of iron- and sulfur-precipitating microbial mats at the Chefren mud volcano (Nile Deep Sea fan, eastern Mediterranean). Appl Environ Microbiol 74:3198–3215

Orcutt B, Boetius A, Elvert M, Samarkin V, Joye SB (2005) Molecular biogeochemistry of sulfate reduction, methanogenesis and the anaerobic oxidation of methane at Gulf of Mexico cold seeps. Geochim Cosmochim Acta 69:4267–4281

Orphan VJ, Hinrichs K-U, Ussler W III, Paull CK, Taylor LT, Sylva SP, Hayes JM, DeLong EF (2001) Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Appl Environ Microbiol 67:1922–1934

Orphan VJ, House CH, Hinrichs K-U, McKeegan KD, DeLong EF (2002) Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proc Natl Acad Sci U S A 99:7663–7668

Pancost RD, Damsté JSS, de Lint S, van der Maarel MJEC, Gottschal JC, the Medinaut shipboard scientific party (2000) Biomarker evidence for widespread anaerobic methane oxidation in Mediterranean sediments by a consortium of methanogenic archaea and bacteria. Appl Environ Microbiol 66:1126–1132

Pernthaler A, Dekas AE, Brown CT, Goffredi SK, Embaye T, Orphan VJ (2008) Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. Proc Natl Acad Sci U S A 105:7052–7057

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WIC, Schouten S, Damsté JSS, Op den Camp HJM, MSM J, Strous M (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440:918–921

Reeburgh WS (1982) A major sink and flux control for methane in marine sediments: anaerobic consumption. In: Fanning K, Mannheim FT (eds) Dynamic environment of the ocean floor. Heath, Lexington, pp 203–217

Reeburgh WS (2007) Oceanic methane biogeochemistry. Chem Rev 107:486–513

Reed AJ, Dorn R, Van Dover CL, Lutz RA, Vetriani C (2009) Phylogenetic diversity of methanogenic, sulfate-reducing and methanotrophic prokaryotes from deep-sea hydrothermal vents and cold seeps. Deep Sea Res Part II Top Stud Oceanogr 56:1665–1674

Reed DC, Deemer BR, van Grinsven S, Harrison JA (2017) Are elusive anaerobic pathways key methane sinks in eutrophic lakes and reservoirs? Biogeochemistry 134:29–39

Reitner J, Peckmann J, Blumenberg M, Michaelis W, Reimer A, Thiel V (2005) Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. Palaegeogr Paleoclimatol Paleoecol 227:181–227

Roland FA, Darchambeau F, Morana C, Bouillon S, Borges AV (2017) Emission and oxidation of methane in a meromictic, eutrophic and temperate lake (Dendre, Belgium). Chemosphere 168:756–764

Ruff, SE, Kuhfuss H, Wegener G, Lott C, Ramette A, Wiedling J, Knittel K, Weber M (2016) Microbial communities of shallow water methane cold seeps off Elba, Mediterranean Sea. Frontiers in Microbiol 7:374

Ruff SE, Biddle JF, Teske AP, Knittel K, Boetius A, Ramette A (2015) Global dispersion and local diversification of the methane seep microbiome. Proc Natl Acad Sci U S A 112:4015–4020

Scheller S, Yu H, Chadwick GL, McGlynn SE, Orphan VJ (2016) Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. Science 351:703–707

Schouten S, Wakeham SG, Hopmans EC, Damsté JSS (2003) Biogeochemical evidence that thermophilic archaea mediate the anaerobic oxidation of methane. Appl Environ Microbiol 69:1680–1686

Schreiber L, Holler T, Knittel K, Meyerdierks A, Amann R (2010) Identification of the dominant sulfate-reducing bacterial partner of anaerobic methanotrophs of the ANME-2 clade. Environ Microbiol 12:2327–2340

Schrenk MO, Kelley DS, Delaney JR, Baross JA (2003) Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. Appl Environ Microbiol 69:3580–3592

Shima S, Thauer RK (2005) Methyl-coenzyme M reductase and the anaerobic oxidation of methane in methanotrophic archaea. Curr Opin Microbiol 8:643–648

Sivan O, Antler G, Turchyn AV, Marlow JJ, Orphan VJ (2014) Iron oxides stimulate sulfate-driven anaerobic methane oxidation in seeps. Proc Natl Acad Sci U S A 111:E4139–E4147

Skennerton CT, Chourey K, Iyer R, Hettich RL, Tyson GW, Orphan VJ (2017) Methane-fueled syntrophy through extracellular electron transfer: uncovering the genomic traits conserved within diverse bacterial partners of anaerobic methanotrophic archaea. MBio 8:e00530–e00517

Stadnitskaia A, Muyzer G, Abbas B, Coolen MJL, Hopmans EC, Baas M, van Weering TCE, Ivanov MK, Poludetkina E, Damsté JSS (2005) Biomarker and 16S rDNA evidence for anaerobic oxidation of methane and related carbonate precipitation in deep-sea mud volcanoes of the Sorokin Trough, Black Sea. Mar Geol 217:67–96

Steen IH, Dahle H, Stokke R, Roalkvam I, Daae F-L, Rapp HT, Pedersen RB, Thorseth IH (2016) Novel barite chimneys at the Loki's Castle vent field shed light on key factors shaping microbial communities and functions in hydrothermal systems. Front Microbiol 6:1510

Stokke R, Roalkvam I, Lanzen A, Haflidason H, Steen IH (2012) Integrated metagenomic and metaproteomic analyses of an ANME-1-dominated community in marine cold seep sediments. Environ Microbiol 14:1333–1346

Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR (2010) Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic *Bacteria*. Science 330:1413–1415

Teske A, Hinrichs K-U, Edgcomb V, de Vera Gomez A, Kysela D, Sylva SP, Sogin ML, Jannasch HW (2002) Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. Appl Environ Microbiol 68:1994–2007

Thauer RK, Shima S (2008) Methane as fuel for anaerobic microorganisms. Ann N Y Acad Sci 1125:158–170

Timmers PHA, Welte CU, Koehorst JJ, Plugge CM, Jetten MSM, Stams AJM (2017) Reverse methanogenesis and respiration in methanotrophic archaea. Archaea 2017:1654237

Trembath-Reichert E, Case DH, Orphan VJ (2016) Characterization of microbial associations with methanotrophic archaea and sulfate-reducing bacteria through statistical comparison of nested Magneto-FISH enrichments. Peer J 4:e1913

Treude T, Krüger M, Boetius A, Jørgensen BB (2005) Environmental control on anaerobic oxidation of methane in the gassy sediments of Eckernf05 Bay (German Baltic). Limnol Oceanogr 50:1771–1786

Treude T, Orphan V, Knittel K, Gieseke A, House CH, Boetius A (2007) Consumption of methane and CO_2 by methanotrophic microbial mats from gas seeps of the anoxic Black Sea. Appl Environ Mcrobiol 73:2271–2283

Valentine DL, Reeburgh WS (2000) New perspectives on anaerobic methane oxidation. Environ Microbiol 2:477–484

Valenzuela EI, Prieto-Davó A, López-Lozano NE, Hernández-Eligio A, Vega-Alvarado L, Juárez K, García-González AS, López MG, Cervantes FJ (2017) Anaerobic methane oxidation driven by microbial reduction of natural organic matter in a tropical wetland. Appl Environ Microbiol 83:e00645–e00617

Walker DJF, Ramesh Y, Adhikari RY, Holmes DE, Ward JE, Woodard TL, Nevin KP, Lovley DR (2017) Electrically conductive pili from pilin genes of phylogenetically diverse microorganisms. ISME J. https://doi.org/10.1038/ismej.2017.141

Wang FP, Zhang Y, Chen Y, He Y, Qi J, Hinrichs KU, Zhang XX, Xiao X, Boon N (2014) Methanotrophic archaea possessing diverging methane-oxidizing and electron-transporting pathways. ISME J 8:1069–1078

Weber HS, Habicht KS, Thamdrup B (2017) Anaerobic methanotrophic archaea of the ANME-2d cluster are active in a low-sulfate, iron-rich freshwater sediment. Front Microbiol 8:619

Wegener G, Shovitri M, Knittel K, Niemann H, Hovland M, Boetius A (2008a) Biogeochemical processes and microbial diversity of the Gullfaks and Tommeliten methane seeps (Northern North Sea). Biogeosciences 5:1127–1144

Wegener G, Niemann H, Elvert M, Hinrichs K-U, Boetius A (2008b) Assimilation of methane and inorganic carbon by microbial communities mediating the anaerobic oxidation of methane. Environ Microbiol 10:2287–2298

Wegener G, Krukenberg V, Riedel D, Tegetmeyer HE, Boetius A (2015) Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. Nature 526:587–590

Wegener G, Krukenberg V, Ruff SE, Kellermann MY, Knittel K (2016) Metabolic capabilities of microorganisms involved in and associated with the anaerobic oxidation of methane. Front Microbiol 7:46

Yoshinaga MY, Lazar CS, Elvert M, Lin Y-S, Zhu C, Heuer VB, Teske A, Hinrichs K-U (2015) Possible roles of uncultured archaea in carbon cycling in methane-seep sediments. Geochem Cosmochem Acta 164:35–52

Zehnder AJB, Brock TD (1979) Methane formation and methane oxidation by methanogenic bacteria. J Bacteriol 137:420–432

Methanotrophy in Acidic Soils, Including Northern Peatlands

8

Tobin J. Verbeke, Svetlana N. Dedysh, and Peter F. Dunfield

Contents

Abstract

Methane oxidizing microorganisms are present and active in diverse acidic environments including peatlands, geothermal areas, and forest soils. Methanotrophic communities in acidic environments have been examined using cultivation-based physiological analyses as well as cultivation-independent molecular approaches, including omic-technologies. Most investigations have focused on moderately acidophilic, aerobic methanotrophs belonging to the phylum *Proteobacteria* that are capable of growth as low as pH 4. However, some *Verrucomicrobia* are capable of oxidizing methane aerobically at pH 1. *Alphaproteobacteria* methanotrophs generally

T. J. Verbeke · P. F. Dunfield (✉)
Department of Biological Sciences, University of Calgary, Calgary, AB, Canada
e-mail: tobinjames.verbeke@ucalgary.ca; pfdunfie@ucalgary.ca

S. N. Dedysh
S. N. Winogradsky Institute of Microbiology, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia
e-mail: dedysh@mail.ru

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_6

dominate the methanotrophic communities in acidic oligotrophic bogs, while *Gammaproteobacteria* methanotrophs are more predominant in minerotrophic fens. The *Verrucomicrobia* methanotrophs appear to be limited to geothermal or sulfidic environments. Recent evidence has suggested that anaerobic methane oxidation may also be important in acidic peatland environments. The known diversity and metabolic potential of aerobic and anaerobic methanotrophs that are active under acidic conditions has advanced in recent years. This chapter will summarize cultivation, molecular ecology, taxonomy, and physiology studies of acidophilic methanotrophs.

Abbreviations

AOM	anaerobic oxidation of methane
FISH	fluorescence *in situ* hybridization
PLFA	phospholipid fatty acid
pMMO	particulate methane monooxygenase
SIP	stable isotope probing
sMMO	soluble methane monooxygenase

1 Introduction

Methanotrophic bacteria play an important role in the biogeochemical cycling of carbon and in mitigating the atmospheric efflux of the potent greenhouse gas methane. While methane-oxidizing communities are found in diverse habitats, some of the most important terrestrial ecosystems globally are acidic in nature (Dedysh 2009; Yu et al. 2017). Acidic terrestrial environments include peatlands, geothermally influenced areas, and many forest soils. Methane oxidation by acidophilic methanotrophs in these environments is known to occur via aerobic and anaerobic processes. Aerobic methanotrophs belong to the *Proteobacteria* and *Verrucomicrobia* phyla, while anaerobic oxidation processes have been attributed to bacteria of the candidate phylum NC10 as well as to some *Archaea*. This chapter outlines the state of our understanding of methanotrophic bacteria in acidic terrestrial environments, specifically focusing on their taxonomy and physiological mechanisms. The focus of the chapter is only on characterizing the methanotrophic microbes active in these environments, rather than on the complex ecological factors controlling net methane fluxes or the contribution of these fluxes to the global methane budget. For such biogeochemical considerations, one is referred to other recent reviews (e.g., Dean et al. 2018).

2 Methanotrophy in Peatlands

Peatlands account for ~30% of the global terrestrial soil carbon pool and represent one of the largest natural sources of atmospheric methane (Gorham 1991). The anaerobic decay of accumulated organic matter leads to the eventual formation of

methane by methanogenic *Archaea*. Atmospheric methane release is mitigated, however, by methane-oxidizing bacteria. Consuming between 10% and 90% of the methane produced (Segers 1998), aerobic methanotrophs in peatlands are found free-living in the upper, oxic-zones of peatland soil or associated with the submerged parts of mosses (Kip et al. 2010). Although this relationship with mosses has occasionally been dubbed "symbiotic" (Raghoebarsing et al. 2005), better terms are probably "moss associated methanotrophy" or "*Sphagnum*-associated methanotrophy," as the nature of the relationship, and its specificity, are not clear. Most research efforts into methane oxidation in peatlands have focused on aerobic processes; however, there is increasing evidence that anaerobic methanotrophs are also important in anoxic zones (Smemo and Yavitt 2007).

Peatlands may be either fens or ombrotrophic bogs. The latter are especially oligotrophic and acidic due to the lack of nutrients and poor buffering capacity. Measured pH values range from 3.0 to 7.0 but are typically below 5 in ombrotrophic bogs. To survive in these harsh conditions, methanotrophs must therefore be acidophilic. To date, methanotrophy in these environments has been attributed to acidophilic alphaproteobacterial and gammaproteobacterial methanotrophs, but not to the extremely acidophilic methanotroph species in the phylum *Verrucomicrobia,* which appear to be absent in these habitats (Tveit et al. 2013; Sharp et al. 2014).

A large fraction of the world's peatlands are *Sphagnum*-dominated areas in northern Russia, which account for about one-half of the world's peat (Smith et al. 2004), as well as similar ecosystems in northern Canada and Alaska. However, an estimated 11% of all peat area and 15–19% of all peat C is sequestered in tropical peatlands, particularly in Southeast Asia (Page et al. 2011). Tropical peatlands are generally also acidic in nature (e.g., Hribljan et al. 2016; Yule et al. 2016). However, research into peatland methanotrophs has to date been highly biased towards northern latitude sites, and these will necessarily be the focus of this review. Efforts to understand methane dynamics in northern wetlands are particularly important given their potential for increased methane emissions as a result of global warming. Surface warming in the Arctic is progressing at nearly twice the rate of the global average temperature increase and has already increased by 3.5 °C compared to the beginning of the twentieth century (Richter-Menge and Mathis 2017).

A variety of experimental techniques have been used to identify acidophilic, methanotrophic communities in peatlands. These include: (i) cultivation studies, (ii) cultivation-independent detection and enumeration using fluorescence *in situ* hybridization, (iii) cultivation-independent analysis of signature phospholipid fatty acids (PLFA), and (iv) cultivation-independent recovery of methanotroph-specific gene sequences and analyses of these with cloning-and-sequencing, microarrays, or high-throughput sequencing (Dumont 2014). Methanotrophs can be identified in 16S rRNA gene sequence read sets, or more specifically via sequencing of genes that encode methane monooxygenase enzymes (MMO), which catalyze the initial reaction in the methane oxidation pathway. MMO exists in both soluble (sMMO) and particulate (pMMO) forms, which are not related evolutionarily. Soluble MMO is not universal to methanotrophs, but specific phylogenetic lineages of methanotrophs possessing it can be investigated via recovery and

amplification of the *mmoX* genes encoding one subunit of sMMO. In contrast, the active-site containing subunit of pMMO encoded by *pmoA* is nearly universal among methanotrophs. The only known aerobic methanotrophs lacking pMMO are strains of the genera *Methylocella* and *Methyloferula*. Phylogenies based on *pmoA* also closely correspond to 16S-rRNA gene-based phylogenies making this an excellent tool for cultivation-independent surveying of methanotrophic communities (Knief 2015). Stable isotope probing (SIP) techniques, particularly ^{13}C-DNA-SIP, are often combined with these identification methods to assess activity of different species.

2.1 Aerobic Methane-Oxidizing *Alphaproteobacteria* in Peatlands

Early studies suggested that methanotroph communities in peat bogs are dominated by the genera *Methylocella*, *Methylocapsa*, and *Methylocystis*, which all belong to the class *Alphaproteobacteria* (Dedysh et al. 2001; Chen et al. 2008a). Cultivation-independent molecular recovery of *pmoA* and *mmoX* genes from various peatlands has repeatedly demonstrated an abundance of *Methylocystis* (McDonald et al. 1997; Morris et al. 2002; Jaatinen et al. 2005; Chen et al. 2008a, b; Siljanen et al. 2011; Putkinen et al. 2012). Chen et al. (2008b) used $^{13}CH_4$ DNA-SIP to demonstrate that *Methylocystis* spp. were the most active methanotrophs in six of eight studied peat bogs ranging from pH 4.2 to 4.9. Recovery of *pmoA* mRNA transcripts of *Methylocystis* from Moor House Peat in the UK also indicated that these bacteria were active (Chen et al. 2008a). Chen et al. (2008a) also detected a predominant group ("MHP" clade) of *pmoA* sequences somewhat related to *Methylocapsa* in *Calluna*-covered moorlands (pH 4.6) of the UK.

In the *Sphagnum-Carex* Bakchar bog (pH 3.6–4.5) of western Siberia, and in a *Sphagnum* peat bog lake (pH 4.2) in Germany, methanotrophs were enumerated using a set of 16S rRNA-targeted FISH probes independently targeting *Methylocella palustris*, *Methylocapsa acidiphila*, *Methylosinus* spp., *Methylocystis* spp., and methanotrophic *Gammaproteobacteria*. The *Alphaproteobacteria* were abundant: *Methylocella palustris* ($>10^6$ cells g^{-1} of wet peat in Bakchar), *Methylocystis* spp. ($>10^6$ cells g^{-1} in both sites), and *Methylocapsa acidiphila* ($>10^5$ cells g^{-1} in both sites), while the *Gammaproteobacteria* accounted for $<1\%$ of the methanotroph populations (Dedysh et al. 2001, 2003).

This trend is supported by cultivation efforts. Most cultivated and taxonomically described methanotrophs obtained from peatlands (Table 1) belong to the class *Alphaproteobacteria* and either the family *Methylocystaceae* (*Methylocystis*) or *Beijerinckiaceae* (*Methylocella*, *Methyloferula*, *Methylocapsa)*. Validated species of *Methylocella* isolated from peat include *Methylocella palustris* (type strain, K) and *Methylocella tundrae* (type strain, T4) (Dedysh et al. 2000, 2004). These strains lack a pMMO and have only a sMMO to catalyze methane oxidation. They can be further differentiated from other proteobacterial methanotrophs by their lack of intracellular membrane stacks. Rather, *Methylocella* spp. have vesicular membrane invaginations that are connected to the cytoplasmic membrane. *Methylocella*

Table 1 Characteristics of isolated, acidophilic methanotrophs from peatland, geothermal, and forest soil environments

Strains[a]	pH range (optimum)	Type of MMO	Intracellular membrane	Carbon fixation pathway	Metabolic capability
Peatlands					
Alphaproteobacteria					
Methylocapsa acidiphila B2	4.2–7.2 (5.0–5.5)	pMMO	Type III: a single membrane stack along one side of the envelope	Serine	Obligate methanotroph
Methylocapsa palsarum NE2	4.1–8.0 (5.2–6.5)	pMMO	Type III: a single membrane stack along one side of the envelope	Serine	Obligate methanotroph
Methylocella palustris K	4.5–7.0 (5.0–5.5)	sMMO	Vesicular membrane invaginations connected to the cytoplasmic membrane	Serine	Facultative methanotroph
Methylocella tundrae T4	4.2–7.5 (5.5–6.0)	sMMO	Vesicular membrane invaginations connected to the cytoplasmic membrane	Serine	Facultative methanotroph
Methylocystis bryophila H2s	4.2–7.6 (6.0–6.5)	pMMO; sMMO	Type II: paired membrane stacks along the cell periphery, parallel to the envelope	Serine	Limited facultative methanotroph[b]
Methylocystis heyeri H2	4.4–7.5 (5.8–6.2)	pMMO; sMMO	Type II: paired membrane stacks along the cell periphery, parallel to the envelope	Serine	Limited facultative methanotroph[b]
Methyloferula stellata AR4	3.5–7.2 (4.8–5.2)	sMMO	Vesicular membrane invaginations connected to the cytoplasmic membrane	Serine; RuBP	Obligate methanotroph
Gammaproteobacteria					
Candidatus Methylospira mobilis	4.2–6.5 (6.0–6.5)	pMMO	Type I: a bundle of vesicular disks	RuMP	Obligate methanotroph
Methylobacter tundripaludum SV96	5.5–7.9	pMMO	Type I: a bundle of vesicular disks	RuMP	Obligate methanotroph
Methylomonas paludis MG30	3.8–7.3 (5.8–6.4)	pMMO	Type I: a bundle of vesicular disks	RuMP	Obligate methanotroph

(*continued*)

Table 1 (continued)

Strains[a]	pH range (optimum)	Type of MMO	Intracellular membrane	Carbon fixation pathway	Metabolic capability
Geothermal environments					
Verrucomicrobia					
"Methylacidimicrobium cyclopophantes" 3B	0.6–3.0 (1.5–3.0)	pMMO	ND	CBB	Obligate methanotroph
"Methylacidimicrobium fagopyrum" 3C	0.6–6.0 (1.5–3.0)	pMMO	Membrane stacks orthogonal to the cytoplasmic membrane	CBB	Obligate methanotroph
"Methylacidimicrobium" sp. LP2A	1.0–5.2 (3.1)	pMMO	ND	CBB	Obligate methanotroph
"Methylacidimicrobium tartarophylax" 4AC	0.5–6.0 (1.0–3.0)	pMMO	ND	CBB	Obligate methanotroph
"Methylacidiphilum fumariolicum" SolV	0.8–5.8	pMMO	Carboxysome-like structures	CBB	Methanotroph; $H_2 + CO_2$
"Methylacidiphilum infernorum" V4	1.0–6.0 (2.0–2.5)	pMMO	Carboxysome-like structures	CBB	Obligate methanotroph
"Methylacidiphilum kamchatkense" Kam1	2.0–5.0 (3.5)	pMMO	Carboxysome-like structures	CBB	Obligate methanotroph
Forest soils					
Alphaproteobacteria					
Methylocapsa aurea KYG	5.2–7.2 (6.0–6.2)	pMMO	Type III: a single membrane stack along one side of the envelope	Serine	Limited facultative methanotroph[b]
Methylocella silvestris BL2	4.5–7.0 (5.5)	sMMO	Vesicular membrane invaginations connected to the cytoplasmic membrane	Serine	Facultative methanotroph
Gammaproteobacteria					
Methylovulum miyakonense HT12	6.0–7.5	pMMO; sMMO	Type I: a bundle of vesicular disks	RuMP	Obligate methanotroph

Abbreviations: *CBB* Calvin–Benson–Basham cycle, *ND* not detected or not reported, *RuBP* ribulose bisphosphate pathway, *RuMP* ribulose monophosphate pathway
[a]Strains have been limited to type strains only
[b]Growth on acetate, in addition to methane/methanol, has been reported

palustris and *Methylocella tundrae* are capable of growth at pH 4.5–7.0 (optimum 5.0–5.5) and pH 4.2–7.5 (optimum 5.5–6.0), respectively. An additional unique characteristic of *Methylocella* spp. is their ability to grow on various compounds containing carbon-carbon bonds in addition to methane. This metabolic capability identified some *Methylocella* spp. as the first facultative methanotrophs and the most

catabolically versatile of all known methanotrophs (Dedysh et al. 2005; Dedysh and Dunfield 2018).

Methyloferula stellata (type strain, AR4) is another species known to encode only sMMO and not pMMO (Vorobev et al. 2011). Isolated from *Sphagnum* bogs in Russia, this methanotroph is moderately acidophilic with an optimum pH for growth between 4.8 and 5.2 (Table 1). Unlike *Methylocella* spp., *Methyloferula stellata* is an obligate methanotroph. It is also reported to fix carbon via the ribulose-bisphosphate pathway in addition to the serine pathway common to most alphaproteobacterial methanotrophs.

Methylocella and *Methyloferula* are among the least well studied of all methanotrophs using cultivation-independent studies, because they lack a pMMO enzyme and therefore cannot be detected via *pmoA* recovery, the method of choice for the majority of microbial ecology studies. Consequently, their importance relative to other species is poorly elucidated. Even detecting these genera via 16S rRNA genes sequencing is problematic, since unlike the *Gammaproteobacteria* methanotrophs or the other *Alphaproteobacteria* methanotrophs in the family *Methylocystaceae*, the methanotrophs within the family *Beijerinckiaceae* are closely related (up to 97% 16S rRNA gene sequence identity) to non-methanotrophs, and it is often difficult to discern if a sequence detected is or is not from a methanotroph. However, *Methylocella*-like *mmoX* sequences have been found in peat ecosystems (Chen et al. 2008a; Gupta et al. 2012) and the genus has been detected via 16S rRNA targeted FISH in some studies (Dedysh et al. 2001, 2003).

Methylocapsa acidiphila B2^T and *Methylocapsa palsarum* NE2^T are moderately acidophilic bacteria isolated from *Sphagnum*-rich environments in Siberia and Norway, respectively (Dedysh et al. 2002, 2015). These strains of *Methylocapsa* are obligate methanotrophs, encode a pMMO enzyme, and have an intracellular membrane system (Table 1). Both strains can grow over a broad pH range, with pH 4.2–7.2 supporting growth for *Methylocapsa acidiphila* and 4.1–8.0 for *Methylocapsa palsarum*.

Most described *Methylocystis* cultures are neutrophilic, but two species, *Methylocystis heyeri* (type strain, H2^T) and *Methylocystis bryophila* (type strain, H2s^T), are moderately acidophilic (Dedysh et al. 2007; Belova et al. 2013). The strains contain both sMMO and pMMO (Table 1), with the latter strain possessing two distinct *pmo* operons. Genomic analyses of *Methylocystis bryophila* S285 has further identified that in addition to two canonical pMMO-encoding operons *pmoCAB*, this specific strain also contains a third, highly divergent *pxmABC*-gene cluster (Fig. 1) (Han et al. 2018). The *pxmABC*-cluster has been identified primarily in gammaproteobacterial methanotrophs and a limited number of alphaproteobacterial methanotrophs, but its functional role has not yet been identified (Tavormina et al. 2011; Han et al. 2018). Some strains of *Methylocystis* have also been shown to have slow but sustained growth on acetate, identifying them as [limited] facultative methanotrophs (Belova et al. 2011).

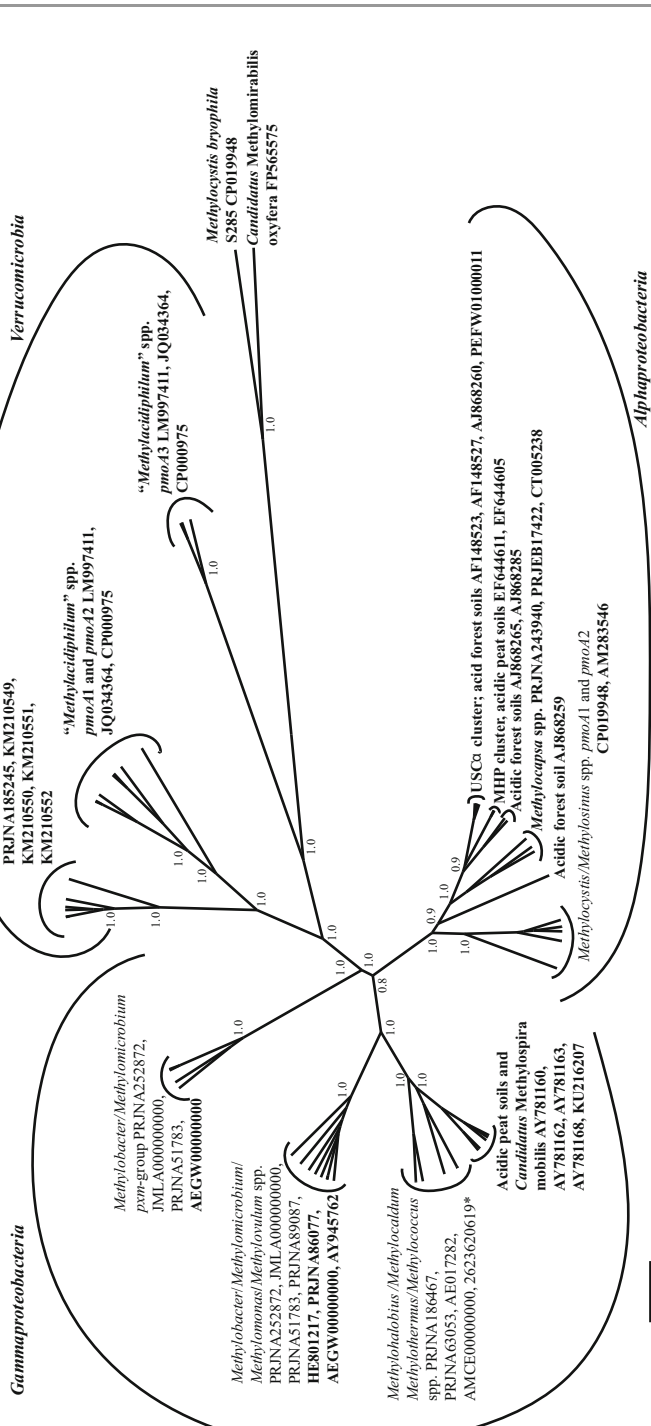

Fig. 1 Phylogenetic analyses comparing acidophilic methanotrophs to select reference strains based on partial *pmoA* gene sequences. Sequences taken from acidophilic isolates or acidic environments are in ***bold***. Sequences used for tree construction were taken from genome sequencing projects, were determined from cultured isolates or via cultivation-independent studies from acidic environments as reported by Holmes et al. (1999), Chen et al. (2008a), Knief et al. (2005, 2006), and Jaatinen et al. (2005), and are identified by their associated GenBank gene/genome accession numbers. The *asterisk* (*) symbol denotes

2.2 Aerobic Methane-Oxidizing *Gammaproteobacteria* in Peatlands

Detection and quantification of a "signature" PLFA of gammaproteobacterial methanotrophs, C16:1ω8c, has been used as evidence that these bacteria are abundant in peatlands (Krumholz et al. 1995; Sundh et al. 1995). However, the subsequent detection of this PLFA in the acidophilic alphaproteobacterial methanotroph *Methylocystis heyeri* means that it can no longer be used as an indicator of any particular group (Dedysh et al. 2007).

However, more recent sequencing studies have provided new insights into the diversity of peat-associated methanotrophic communities and indicated an important role for *Gammaproteobacteria*. Using a *pmoA*-based microarray to investigate a *Sphagnum* peat bog (pH 3.8–4.3) in the Netherlands, Kip et al. (2011a) showed a high abundance of both *Alphaproteobacteria* (*Methylocystis*, *Methylosinus*) and *Gammaproteobacteria* (*Methylobacter*, *Methylomonas*, *Methylomicrobium*) methanotrophs. Additional support for the prevalence of both lineages was provided by pyrosequencing *pmoA* amplicons from the same environment (Kip et al. 2011a). Gammaproteobacterial reads comprised 58% of the entire dataset, while 40% of the reads could be mapped to *Alphaproteobacteria*. In an independent study that combined metagenomics with sequencing analyses of *pmoA* cDNA amplicons, Esson et al. (2016) also discovered that methanotrophic communities in an acidic peat bog (pH 3.5–4.0) were co-dominated by *Methylocystis* and Methylomonas. *Sphagnum* mosses from three alpine bogs in Austria were found to support a combination of *Methylomonas* and *Methylocystis* as moss-associated methanotrophs (Bragina et al. 2013).

Other studies have even detected a marked predominance of *Gammaproteobacteria* methanotrophs in some sites. Species related to *Methylobacter tundripaludum* and *Methylobacter psychrophilus* were detected as the major methanotrophs in two mildly acidic Arctic fens at pH 5–6 via pyrosequencing of 16S rRNA genes and analysis of mRNA transcripts (Tveit et al. 2013, 2014). While multiple factors can contribute to the structural dynamics of methanotrophic communities in different sites, these studies provide evidence that both gammaproteobacterial and alphaproteobacterial methanotrophs can be important.

Compared to the *Alphaproteobacteria*, fewer isolates of acidophilic methanotrophs from the *Gammaproteobacteria* are available, and most show only a mildly acidophilic phenotype. Isolated from mossy Arctic soil, *Methylobacter psychrophilus* Z-0021^T was one of the first cultivated psychrophilic methanotrophs (Omelchenko et al. 1996; Tourova et al. 1999). *Methylobacter*

Fig. 1 (continued) sequences taken from the Integrated Microbial Genomes database with the associated IMG Genome ID number. Sequences were aligned using Clustal Omega (Sievers et al. 2011) and the tree constructed via Bayesian analyses using the BEAST2 software package (Bouckaert et al. 2014) with the general-time-reversible substitution model for ten million generations sampling every 1000th tree. Posterior probability values of major nodes are shown

psychrophilus-like strains have been detected in mildly acidic fens (Tveit et al. 2013, 2014), while other environmental studies have suggested a greater predominance of these strains in pH-neutral Arctic environments (Berestovskaya et al. 2002; Martineau et al. 2010). The latter observations coincide with the growth characteristics reported for the type strain, which include a pH range of 5.9–7.6 with an optimum of 6.7 (Trotsenko and Khmelenina 2005). Unfortunately, the original culture of Z-0021^T has been lost (Graef et al. 2011). A closely related strain, *Methylobacter tundripaludum* SV96^T, was isolated from a mildly acidic, wetland soil from Svalbard (Wartiainen et al. 2006) and has been reported to grow well from pH 5.5 to 7.9. Using meta-transcriptomics, Tveit et al. (2015) identified a high abundance of pMMO and SSU rRNA transcripts that most closely matched to *Methylobacter tundripaludum* sequences in Arctic peat soils and suggested these strains were the dominant active methanotrophs. Graef et al. (2011) also identified that strains of *Methylobacter*, namely, *Methylobacter tundripaludum*, dominated the methanotrophic community found in a high Arctic wetland. *Methylobacter tundripaludum* and related strains seem to be well adapted to methanotrophy in cold-environments, which can include neutral and mildly acidic habitats. A draft genome of strain SV96^T was also released in 2011 (Svenning et al. 2011). A notable outcome of the resulting analyses was the identification of a *pxm*-version of the MMO-encoding operon in addition to the canonical *pmoCAB* operon encoded within the genome (Fig. 1).

Kip et al. (2011b) reported the growth of cultures of *Methylomonas* and *Methylovulum*-like methanotrophs from peat that were capable of growth as low as pH 3.5–4.1; however, these have never been taxonomically characterized and validated. Danilova et al. (2013) recently described the first *Methylomonas* species from an acidic (pH 3.9) *Sphaghum* peat bog. *Methylomonas paludis* MG30^T has an optimum pH between 5.8 and 6.4, possesses a pMMO, and is an obligate methanotroph capable of growing only on methane and methanol (Table 1). Recently a spiral-shaped methanotroph related to the genera *Methylocaldum* and *Methylococcus* (sometimes called the type Ib methanotrophs) was enriched from *Sphagnum* peat and dubbed *"Candidatus* Methylospira mobilis" (Danilova et al. 2016b). Although its optimum pH is only 6.0–6.5, it is capable of growth down to pH 4.2. This species appears to be widespread in acidic peat bogs based on *pmoA* analysis (Danilova et al. 2016a, b).

2.3 Are There Patterns to Methanotroph Community Structure Across Peatlands?

Clearly, many different methanotroph species, both *Gammaproteobacteria* and *Alphaproteobacteria*, have been identified as predominantly abundant, or predominantly active, in different peatlands. Extrapolating general patterns is complicated not only because every site is unique, but also because nearly every study is also unique and employs a different set of methods. Communities may be identified based on 16S rRNA, *pmoA,* and/or *mmoX* genes, and the actual DNA sequence

variant identifications may be made through denaturing gradient gel electrophoresis, FISH, cloning and sequencing, microarray analysis, or high-throughput sequencing. Some studies include a functional analysis of active species using SIP or transcriptome analyses. Add to this a host of other differences in sampling time and intensity, sample handling, DNA extraction methods, PCR protocols, etc., and comparison becomes somewhat problematic.

Nevertheless, some general trends do emerge. Comparatively nutrient-rich, only mildly acidic (pH >5) fens often show a strong predominance of *Gammaproteobacteria* methanotrophs (Jaatinen et al. 2005; Tveit et al. 2013, 2014; Christiansen et al. 2014), whereas oligotrophic, highly acidic (pH <5) ombrotrophic bogs are more likely to show a strong predominance of *Alphaproteobacteria* methanotrophs (Dedysh et al. 2001; Chen et al. 2008b; Gupta et al. 2012; Kravchenko et al. 2015). Many sites are intermediate and contain both groups.

Studies that simultaneously compare different sites with the same methods are particularly useful when drawing this conclusion, and the pattern of increased *Alphaproteobacteria* methanotroph dominance with increasing acidity and nutrient limitation is surprisingly consistent in these studies. Gupta et al. (2012) compared a nutrient-rich sedge fen to a nutrient-poor *Sphagnum* bog using a combination of ^{13}C-DNA SIP and fingerprinting via *pmoA* and *mmoX*. The bog was predominated by *Alphaproteobacteria* methanotrophs (*Methylocystis*, *Methylosinus*, and *Methylocella*), whereas both *Alphaproteobacteria* and *Gammaproteobacteria* methanotrophs were present and active in the fen. Comparison of a pristine bog (pH 3.8) to adjacent drained sites (pH 4.2–4.8), via *pmoA* gene sequencing, indicated that *Methylocystis* were dominant in the pristine site, while *Methylobacter* became more abundant in the drained site (Kravchenko et al. 2015). In a comparison of a pristine ombrotrophic bog (pH 4.4–4.5) with a minerotrophic fen (pH 4.9–5.2), *Gammaproteobacteria* methanotrophs were detected as the only methanotrophs in the nutrient-rich fen, whereas in the ombrotrophic bog *Methylocystis* were detected along with the *Gammaproteobacteria* (Jaatinen et al. 2005). A comparison of hillock and hollow features of a Siberian bog was made with pyrosequencing of 16S rRNA genes (Grodnitskaya et al. 2018). The hillocks were notably more acidic and nutrient-poor and were dominated by *Alphaproteobacteria* methanotrophs (*Methylosinus* and *Methylocapsa*). In the hollows, both *Gammaproteobacteria* and *Alphaproteobacteria* methanotrophs were detected (Grodnitskaya et al. 2018).

In a more extensive survey, Putkinen et al. (2014) combined *pmoA* microarray studies with *pmoA* and 16S rRNA gene SIP analysis of 17 peatlands that represented a chronosequence of successional stages during the development of a wet meadow into a minerotrophic fen and finally an ombrotrophic bog. They observed a pattern whereby the *Alphaproteobacteria* methanotrophs (*Methylocystis, Methylosinus, Methylocella*, and *Methylocapsa*), plus the *Gammaproteobacteria* methanotroph *"Ca.* Methylospira mobilis," were most active in the late-stage ombrotrophic bogs that had the lowest nutrient availability and pH. Other *Gammaproteobacteria* (esp. *Methylobacter* and *Methylomonas*) methanotrophs were active at all sites, but particularly in the earlier fen stages.

Putkinen et al. (2014) interpreted their results as a consequence of their differing ecological life strategies of the different methanotrophic groups. The trend was consistent with early theories of the niche differentiation of *Gammaproteobacteria* versus *Alphaproteobacteria* methanotrophs, which proposed that *Gammaproteobacteria* methanotrophs thrive in favorable habitats selecting for a high growth rate, or are r-selected. Conversely, the *Alphaproteobacteria* are better suited to more stressful, less optimal habitats, and represent K-selected, or more appropriately L-selected or stress-tolerant bacteria (Hanson and Hanson 1996; Ho et al. 2013; Knief 2015). Ho et al. (2013) have summarized methanotroph strategies using a similar three-member system of Competitor, Stress tolerator, or Ruderal. Both of these classification systems are consistent with some known physiological properties that distinguish the two methanotroph groups. For the *Alphaproteobacteria* methanotrophs, these include: the ability to survive under lower methane concentrations (Knief and Dunfield 2005), a higher prevalence of nitrogenase and the common formation of cysts (Hanson and Hanson 1996), and an ability to grow in extremely low nutrient, oligotrophic media, which is a defining characteristic of all *Beijerinckiaceae* methanotrophs, including *Methylocella* and *Methylocapsa* (Dedysh et al. 2000, 2004, 2015; Vorobev et al. 2011). Conversely the *Gammaproteobacteria* methanotrophs have a more efficient C fixation pathway and generally higher growth rates (Hanson and Hanson 1996) and tend to respond more positively to nitrogen fertilization (Ho et al. 2013). The generalization that all *Gammaproteobacteria* methanotrophs are r-selected and all *Alphaproteobacteria* methanotrophs are L-selected is certainly an oversimplification and should not be applied to all members of either group. Nevertheless, as a general rule governing the distribution of species in ombrotrophic bogs versus minerotrophic fens, there appears to be some truth in it.

2.4 Anaerobic Methane Oxidation in Peatlands

There is increasing evidence of the important role that anaerobic methane oxidation (AOM) plays in peatlands. More than a decade ago, Smemo and Yavitt (2007) found that AOM can occur simultaneously with methanogenesis in a variety of peatlands and can consume large amounts of the methane produced. More recently, Gupta et al. (2013) confirmed that AOM can be widespread across diverse latitudes and peatland type. In this study, AOM was observed in both fen and bog wetlands at pH values ranging from 3.6 to 5.9. Despite increasing amounts of flux-based evidence, mechanistic insights into this process have just begun to appear.

AOM is now known to be coupled to a variety of possible electron acceptors other than molecular oxygen. In marine environments, anaerobic methanotrophic (ANME) *Archaea* can form synergistic consortia with sulfate-reducing bacteria allowing for methane to be oxidized using sulfate as the terminal electron acceptor. Other terminal electron acceptors like iron, manganese, nitrite, and nitrate have been reported to be used in methane oxidation and should be more thermodynamically favorable than

Table 2 Reactions and associated free energies under standard conditions for possible terminal electron acceptors relevant to methane oxidation

Reaction	ΔG^O (kJ mol^{-1} CH_4)[a]
$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	−842.3
$CH_4 + SO_4^{2-} + 2H^+ \rightarrow CO_2 + H_2S + 2H_2O$	−92.8
$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O$	−503.4
$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O$	−929.0
$CH_4 + 8Fe^{3+} + 2H_2O \rightarrow CO_2 + 8Fe^{2+} + 8H^+$	−454.6
$5CH_4 + 8MnO_4^- + 24H^+ \rightarrow 5CO_2 + 8Mn^{2+} + 22H_2O$	−1028.1

[a]Values as reported in Caldwell et al. (2008) or Welte et al. (2016)

sulfate-coupled processes (Table 2) (Beal et al. 2009; Smemo and Yavitt 2011; Zhu et al. 2012; Ettwig et al. 2016). Assessment of AOM processes in peatlands using these electron acceptors is still somewhat limited, however. Peatlands are often considered metal- and nutrient-poor environments that, with a few exceptions, also generally have low concentrations of sulfate and nitrate, making the use of these alternative acceptors improbable (Smemo and Yavitt 2011). An alternative hypothesis is that AOM-catalyzing microbes use humic acids to shuttle electrons to metal-reducing organisms or to deeper anoxic peat (Smemo and Yavitt 2011). Humic substances are known to transfer electrons under anoxic conditions, and this process would be plausible given the organic-rich nature of peatlands, but the involvement of this mechanism in AOM-processes has not yet been explored.

Nitrite-dependent AOM has been reported in a *Sphagnum*-dominated peatland fed by nitrate-enriched groundwater (Zhu et al. 2012). Analyses of porewater samples found that nitrate and methane had depth counter-gradients with nitrate decreasing at lower depths, while methane concentrations increased. A transition zone where both compounds were depleted was identified. This transition region correlated with an increased abundance of the bacterium *"Candidatus* Methylomirabilis oxyfera" – an organism proposed to catalyze nitrate/nitrite-dependent methane oxidation. Zhu et al. (2012) further showed that enrichment cultures could achieve nitrite-dependent methane oxidation across a pH range from 5.9 to 7.5 identifying the acidophilic potential of this process. Additional support for the involvement of "*Candidatus* Methylomirabilis oxyfera" in nitrite-dependent AOM was later provided in a study linking stable-isotope analyses to bacterium-specific 16S rRNA gene-qPCR primers in both acidic and neutral wetlands (Hu et al. 2014).

In Candidatus "Methylomirabilis oxyfera"-like cultures, methane oxidation is proposed to occur via canonical aerobic methane oxidation pathways. This pathway is proposed despite the observation that methane is oxidized under anaerobic conditions and evidence that methane and nitrite conversion rates decrease at elevated O_2 concentration (Luesken et al. 2012). Instead of consuming O_2 from the surrounding environment, the molecular oxygen needed for methane oxidation may be produced by the bacterium through the dismutation of nitric oxide into dinitrogen gas and O_2 (Zhu et al. 2012).

Nitrate-driven AOM has also been reported for a specific ANME-archaeal lineage (ANME-2d) described as "*Candidatus* Methanoperedenaceae" (Haroon et al. 2013). Unlike Candidatus Methylomirabilis oxyfera-like organisms, this lineage is proposed to catalyze methane-oxidation via reverse methanogenesis with the initial reaction catalyzed by methyl-CoM reductase. Additionally, this lineage is not thought to be capable of AOM independently and is instead reliant on the syntrophic feeding of nitrite to an ammonium-oxidizing bacterium (Haroon et al. 2013). It is important to note, however, that while reverse methanogenesis in peatlands is considered possible (Blazewicz et al. 2012), the experiments used to describe the ANME-2d mechanisms of AOM were done in neutrophilic bioreactors.

3 Geothermally Influenced Environments

Geothermal environments are characterized by high temperatures and are frequently also acidic due to the oxidation of sulfur compounds. While methane concentrations in geothermal gas are typically <1%, some geothermal systems can have molar fractions of methane between 1% and 11% (v/v gas) with anomalies up to 27% (Giggenbach 1995; Etiope and Klusman 2002). The first evidence of extremely acidic methane oxidation was observed in geothermal soils in the Solfatara volcano region near Naples, Italy, a site characterized by high temperature (50–95 °C) and pH as low as 1.0 (Castaldi and Tedesco 2005). Later, atmospheric methane release in steaming geothermal surface soils in New Zealand was found to be mitigated by methanotrophic bacteria (Dunfield et al. 2007). In 2007, cultured isolates were simultaneously reported from three acidic geothermal sites, including an acidic hot spring in Kamchatka, Russia (isolate Kam1), the Solfatara volcano region (isolate SolV) and steaming soil at Tikitere, New Zealand (isolate V4) (Dunfield et al. 2007; Pol et al. 2007; Islam et al. 2008). These isolates had a pH optimum of 2.0–3.5 with a lower limit of 0.8 (Table 1) and could grow at temperatures up to 65 °C. Based on 16S rRNA gene analyses, all three isolates formed a single genus-level cluster within the *Verrucomicrobia* phylum, identifying them as the first methanotrophs outside of the *Proteobacteria*. This cluster is currently described as "*Methylacidiphilum*," but this taxonomic designation remains to be validated.

A second, not-yet validated genus of methanotrophic *Verrucomicrobia*, called "*Methylacidimicrobium,*" has more recently been described (Sharp et al. 2014; van Teesling et al. 2014). This genus is comprised of mesophilic isolates from the Solfatara crater (Naples, Italy) and a geothermal soil in New Zealand. The demonstration that both mesophilic and thermophilic strains of methanotrophic *Verrucomicrobia* exist suggests this phylotype may be more widespread than presently known.

Putative methanotrophic *Verrucomicrobia* were found in several environments over a wide temperature range (22.5–81.6 °C) in New Zealand (Sharp et al. 2014). However, Sharp et al. (2014) detected them only in natural acidic environments (pH <5) that were geothermally influenced, and not in acidic bogs. Evidence for

these bacteria has also been identified by 16S-rDNA sequence analyses in sulfide-corroded sewage pipes (Pagaling et al. 2014), as well as at multiple depths in a nitrogen-fertilized paddy soil (Vaksmaa et al. 2017). In the latter case, however, the sequences shared only 85% nucleotide sequence identity with cultured representatives of the "*Methylacidiphilum*" group, and the involvement of these bacteria in methane oxidation should be viewed very cautiously pending further investigation.

With the exception of a few facultative methanotrophs, methane-oxidizing bacteria have limited metabolic versatility (Dunfield and Dedysh 2014). Recent investigations involving "*Methylacidiphilum*" strains are now challenging our understanding of these niche limitations in methanotrophic *Verrucomicrobia*, however. Isolate SolV has been reported to grow on hydrogen and carbon dioxide in the absence of methane (Mohammadi et al. 2017), which is mediated by two distinct uptake hydrogenases. The two hydrogenases differ in their sensitivity towards oxygen, and RNA-seq analyses have shown differences in expression levels of the encoding genes as a function of oxygen concentration. Carere et al. (2017) have further shown that "*Methylacidiphilum*" sp. RTK17.1 is capable of mixotrophic growth, whereby methane and H_2 can be consumed simultaneously to support respiration and carbon fixation. Given these new insights into the metabolic versatility of some of these strains, the number of possible ecological niches occupied by this group of methanotrophs may be larger than currently recognized.

Like their aerobic, proteobacterial counterparts, the first reaction in methane oxidation by methanotrophic *Verrucomicrobia* is catalyzed by MMO enzyme complexes. Genomic analyses of cultivated "*Methylacidimicrobium*" and "*Methylacidiphilum*" species have only identified pMMO encoding genes and no sMMO-encoding homologues have been identified (Pol et al. 2007; Hou et al. 2008; Anvar et al. 2014; Sharp et al. 2014; van Teesling et al. 2014; Erikstad and Birkeland 2015). Strains in the genus "*Methylacidiphilum"* contain three complete *pmoCAB* operons, which are known to vary from each other by up to 50% in amino acid sequence (Fig. 1). In silico analyses have provided evidence that one of these operons, *pmoCAB*3, has been obtained through lateral gene transfer rather than paralogous replication (Sharp et al. 2013). In contrast, "*Methylacidimicrobium*" strains have only a single *pmoCAB* operon, except strain LP2A, which has two, near-identical operons (Fig. 1). "*Methylacidiphilum kamchatkense"* Kam 1 and "*Methylacidimicrobium*" sp. LP2A genomes also encode orphan *pmoC* genes, but the role of these orphan genes, if any, is not yet clear.

"*Methylacidimicrobium"* strain 3C is the only strain of either genus known to contain intracytoplasmic membrane stacks, which is a trait found in pMMO-encoding proteobacterial methanotrophs (Table 1). Rather, "*Methylacidiphilum"* species are known to contain carboxysome-like compartments. These structures may serve to anchor pMMO enzymes, but further investigation to confirm this hypothesis is required. Alternatively, they may simply play a role as glycogen storage vesicles (Khadem et al. 2012). *Verrucomicrobia* methanotrophs fix carbon dioxide via the Calvin-Benson-Basham cycle, rather than assimilate it via the ribulose-monophosphate or serine pathways found in gammaproteobacterial and alphaproteobacterial methanotrophs, respectively (Table 1).

4 Other Soils

Many terrestrial soils and sediments are mildly acidic. A thorough examination of the methanotrophs in all such sites would be well beyond the scope of this brief review. Nevertheless, some mention should be made of the putative USCα group of proposed atmospheric methane oxidizers present in primarily acidic forest soils.

Estimates of global methane uptake by oxic, well-aerated upland soil vary widely, but current estimates fall within the range of 9–47 Tg C^{-1} $year^{-1}$ (Ciais et al. 2013). Of these, forest soils are estimated to consume ~2.5 times more methane globally than grassland soils (Yu et al. 2017). The source of methane for these populations is the overlying atmosphere, where globally averaged mixing ratios are ~1.83 ppmv (Yu et al. 2017). At these low levels, and with the realization that most forest soils are moderately acidic (pH 4–6), it stands to reason that methanotrophic populations in forest soils are both acidophilic and oligotrophic. Kinetic responses for methane uptake in forest soils follow a typical Michaelis-Menten curve in response to methane concentration, but the apparent affinity is orders of magnitude higher in soils (10–100 nM) than in methanotrophic cultures (1–10 μM). This suggests that the methanotrophs in these soils possess a high-affinity version of MMO allowing them to survive on trace levels of atmospheric methane (Knief et al. 2006)

The cultivation of these "high-affinity" methane oxidizers found in upland soils has not yet been successful. Holmes et al. (1999) were the first to describe atmospheric methane-oxidizing bacterial communities in three acidic forest soils (pH 3.4–4.9). Incubation of the soils under an atmosphere of $^{14}CH_4$ at low concentrations (<50 ppmv) allowed for the ^{14}C-labelled phospholipid fatty acid (PLFA) profiles of the "high-affinity" methanotrophic populations to be determined. The recovered PLFAs were most similar to acidophilic alphaproteobacterial methanotrophs, namely, *Methylocapsa* spp. Based on sequence phylogeny, Holmes et al. (1999) also discovered that *pmoA* sequences retrieved from the soils formed a distinct clade from known methanotrophs and only showed 80% amino acid identity to their closest relative, *Methylocapsa acidiphila* (also see Fig. 1). This clade of *pmoA* sequences was originally designated RA14 but is now most often referred to as upland soil cluster alpha: USCα (Knief and Dunfield 2005).

With no cultured representatives, insights into the genomic and metabolic potential of the USCα clade were previously limited to a 42-kb fosmid clone that contained the key genes for methane oxidation (Ricke et al. 2005). Recently, however, targeted cell enrichments of an acidic (pH ~4) forest soil in Germany combined with metagenomic analyses have allowed for the reconstruction of a draft genome of a USCα clade member (Pratscher et al. 2018). Binning of multiple metagenomes based on tetranucleotide frequency and abundance identified a USCα bin that also contained a partial 16S rRNA gene sequence. The 16S-rRNA sequence showed 96% sequence identity to *Methylocapsa palsarum* NE2 and *Methylocapsa aurea* KYG, the two most closely related cultivated strains. In agreement with previous findings, the *pmoA* sequence also clustered with sequences from *Methylocapsa*, suggesting a close evolutionary relationship between these groups. In analyzing the reconstructed genome, Pratscher et al. (2018) identified only a single

pmoCAB operon, no sMMO and proposed that carbon assimilation occurs primarily via the serine cycle. Enzymes supporting acetate utilization via the glyoxylate cycle were further identified in the draft genome, suggesting members of USCα may be facultative methanotrophs.

Multiple alphaproteobacterial methanotrophs have now been isolated from forest soil (Table 1). One of the first was *Methylocella silvestris* BL2, which was isolated from an acidic (pH ~4.0) forest cambisol near Marburg, Germany. Like other *Methylocella*, this bacterium lacks intracellular membranes and a pMMO but encodes an sMMO (Dunfield et al. 2003). It is also capable of facultative growth as it grows on methylamine, acetate, pyruvate, succinate, malate, ethanol, and methanol in addition to methane (Dedysh et al. 2005). Two other strains, *Methylocapsa aurea* KYG and *Methyloferula stellata* LAY, were also isolated from acidic forest soils in Germany (Dunfield et al. 2010; Vorobev et al. 2011). Like *Methylocella silvestris* BL2, *Methylocapsa aurea* KYG is a facultative methanotroph capable of weak growth on acetate (Dunfield et al. 2010). Unlike *Methylocella* strains, however, *Methylocapsa aurea* KYG has a pMMO and intracellular membranes, but no sMMO. *Methyloferula stellata* LAY has only an sMMO. The three strains can be further differentiated by their pH optima which are 5.5, 6.0–6.2, and 4.8–5.2 for *Methylocella silvestris*, *Methylocapsa aurea,* and *Methyloferula stellata,* respectively.

One of the first gammaproteobacterial methanotrophs isolated from forest soil was *Methylovulum miyakonense* HT12 (Iguchi et al. 2010). Isolated from a forest soil in Japan, this methanotroph is neutrophilic with a growth range of pH 6.0–7.5. An obligate methanotroph, *Methylovulum miyakonense,* has both particulate and soluble versions of MMO (Iguchi et al. 2010; Hamilton et al. 2015) and assimilates carbon via the ribulose monophosphate pathway (Table 1). The bacterium also lacks unsaturated C_{16}-fatty acids, which are typical of most type-I methanotrophs.

Two distinct bacterial strains, BFH1 and BFH2, have also been isolated from tropical topsoil in Bangladesh (Islam et al. 2016). Both strains are capable of growth at pH 4.2–7.5 and can grow at temperatures up to 60 °C. Genes for *pmoA* were detected, but *mmoX* was absent. Analysis of the 16S gene sequence suggests that these isolates represent a novel genus in the family *Methylococcaceae*, but these strains have not yet been described taxonomically.

5 Future Prospects and Research Needs

Conventional and omic-technologies have provided insights into community structure-function relationships in acidophilic, methane-consuming environments. Continued research into (i) the relative contribution of different species to methane uptake and (ii) a better understanding of the factors that control dominance by distinct lineages is warranted. A comparative analysis of the methanotrophs in tropical peatlands would also be valuable to compare with the large number of studies undertaken in northern latitudes.

There is a particular need to elucidate the anaerobic methane-oxidizers. A lack of cultivated representatives capable of AOM has hindered physiological analyses of this group as well as an accurate assessment of their methane-uptake potential. While mechanisms for AOM have begun to emerge (Table 2), many of these require additional confirmatory evidence made possible only through axenic-culture experimentation. Improved understanding of the potential for environmentally relevant, non-O_2 electron acceptors (or shuttles such as humic acids in peat) to contribute to methane oxidation would be of tremendous value in understanding acidophilic methane consumption through AOM-processes.

Two consistent themes emerge in efforts to better understand global methanotrophic processes, particularly in regards to acidophilic methanotrophy. These include the continued exploration of the diversity of methanotrophic bacteria and an improved understanding of the factors that limit niche expansion. For example, the study by Sharp et al. (2014) provided an assessment of the distribution of verrucomicrobial methanotrophs in Canada and New Zealand. Expansion of this study to other regions would be of value and could possibly provide insights into why verrucomicrobial methanotrophs seem limited to geothermally-influenced soils. Similarly, the factors limiting cultivation of USCα members remain unknown. Continued exploration and technological advancements may soon provide novel strategies for cultivation, but insights into the metabolic potential of this globally important group will remain somewhat elusive until this can be achieved. By developing a better understanding of the phylogenetic and physiological diversity of acidophilic methanotrophs, it will allow for improved understanding of how these environments contribute to the global methane cycle now and in the future.

References

Anvar SY, Frank J, Pol A, Schmitz A, Kraaijeveld K, den Dunnen JT, Op den Camp HJM (2014) The genomic landscape of the verrucomicrobial methanotroph *Methylacidiphilum fumariolicum* SolV. BMC Genomics 15:914. https://doi.org/10.1186/1471-2164-15-914

Beal E, House C, Orphan VJ (2009) Manganese- and iron-dependent marine methane oxidation. Science 325:184–187. https://doi.org/10.1126/science.1169984

Belova SE, Baani M, Suzina NE, Bodelier PL, Liesack W, Dedysh SN (2011) Acetate utilization as a survival strategy of peat-inhabiting *Methylocystis* spp. Environ Microbiol Rep 3:36–46. https://doi.org/10.1111/j.1758-2229.2010.00180.x

Belova SE, Kulichevskaya IS, Bodelier PL, Dedysh SN (2013) *Methylocystis bryophila* sp. nov., a facultatively methanotrophic bacterium from acidic *Sphagnum* peat, and emended description of the genus *Methylocystis* (ex Whittenbury et al. 1970) Bowman et al. 1993. Int J Syst Evol Microbiol 63:1096–1104. https://doi.org/10.1099/ijs.0.043505-0

Berestovskaya YY, Vasilieva L, Chestnykh O, Zavarzin GA (2002) Methanotrophs of the psychrophilic microbial community of the Russian Arctic tundra. Mikrobiologiia 71:460–466

Blazewicz SJ, Petersen DG, Waldrop MP, Firestone MK (2012) Anaerobic oxidation of methane in tropical and boreal soils: ecological significance in terrestrial methane cycling. J Geophys Res Biogeosci 117:G02033. https://doi.org/10.1029/2011jg001864

Bouckaert R, Heled J, Kuhnert D, Vaughan T, Wu C, Xie D, Suchard MA, Rambaut A, Drummond A (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput Biol 10:e1003537. https://doi.org/10.1371/journal.pcbi.1003537.g001

Bragina A, Berg C, Muller H, Moser D, Berg G (2013) Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. Sci Rep 3:1955. https://doi.org/10.1038/srep01955

Caldwell S, Laidler J, Brewer E, Eberly J, Sandborgh S, Colwell F (2008) Anaerobic oxidation of methane: mechanisms, bioenergetics, and the ecology of associated microorganisms. Environ Sci Technol 42:6791–6799. https://doi.org/10.1021/es800120b

Carere CR, Hards K, Houghton KM, Power JF, McDonald B, Collet C, Gapes DJ, Sparling R, Boyd ES, Cook GM, Greening C, Stott MB (2017) Mixotrophy drives niche expansion of verrucomicrobial methanotrophs. ISME J 11:2599–2610. https://doi.org/10.1038/ismej.2017.112

Castaldi S, Tedesco D (2005) Methane production and consumption in an active volcanic environment of Southern Italy. Chemosphere 58:131–139. https://doi.org/10.1016/j.chemosphere.2004.08.023

Chen Y, Dumont MG, McNamara NP, Chamberlain PM, Bodrossy L, Stralis-Pavese N, Murrell JC (2008a) Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP–PLFA analyses. Environ Microbiol 10:446–459. https://doi.org/10.1111/j.1462-2920.2007.01466.x

Chen Y, Dumont MG, Neufeld JD, Bodrossy L, Stralis-Pavese N, McNamara NP, Ostle N, Briones MJ, Murrell JC (2008b) Revealing the uncultivated majority: combining DNA stable-isotope probing, multiple displacement amplification and metagenomic analyses of uncultivated *Methylocystis* in acidic peatlands. Environ Microbiol 10:2609–2622. https://doi.org/10.1111/j.1462-2920.2008.01683.x

Christiansen JR, Romero AJB, Jørgensen NOG, Glaring MA, Jørgensen CJ, Berg LK, Elberling B (2014) Methane fluxes and the functional groups of methanotrophs and methanogens in a young Arctic landscape on Disko Island, West Greenland. Biogeochemistry 122:15–33. https://doi.org/10.1007/s10533-014-0026-7

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, Jones C, Le Quere C, Myneni R, Piao S, Thornton P (2013) Carbon and other biogeochemical cycles. In: Heinze C, Tans P, Vesala T (eds) Climate change 2013: the physical science basis. Intergovernmental panel on climate change. Cambridge University Press, Cambridge, pp 465–570

Danilova OV, Kulichevskaya IS, Rozova ON, Detkova EN, Bodelier PL, Trotsenko YA, Dedysh SN (2013) *Methylomonas paludis* sp. nov., the first acid-tolerant member of the genus *Methylomonas*, from an acidic wetland. Int J Syst Evol Microbiol 63:2282–2289. https://doi.org/10.1099/ijs.0.045658-0

Danilova OV, Belova SE, Gagarinova IV, Dedysh SN (2016a) Microbial community composition and methanotroph diversity of a subarctic wetland in Russia. Mikrobiologiia 85:583–591. https://doi.org/10.1134/s0026261716050039

Danilova OV, Suzina NE, Van De Kamp J, Svenning MM, Bodrossy L, Dedysh SN (2016b) A new cell morphotype among methane oxidizers: a spiral-shaped obligately microaerophilic methanotroph from northern low-oxygen environments. ISME J 10:2734–2743. https://doi.org/10.1038/ismej.2016.48

Dean JF, Middelburg JJ, Röckmann T, Aerts R, Blauw LG, Egger M, Jetten MSM, de Jong AEE, Meisel OH, Rasigraf O, Slomp CP, in't Zandt MH, Dolman AJ (2018) Methane feedbacks to the global climate system in a warmer world. Rev Geophys. https://doi.org/10.1002/2017rg000559

Dedysh SN (2009) Exploring methanotroph diversity in acidic northern wetlands: molecular and cultivation-based studies. Microbiology 78:655–669. https://doi.org/10.1134/s0026261709060010

Dedysh SN, Dunfield PF (2018) Facultative methane oxidizers. In: TJ (ed) Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology, Handbook of Hydrocarbon and Lipid Microbiology, Springer Nature, Switzerland. https://doi.org/10.1007/978-3-319-60063-5_6-1

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 50:955–969. https://doi.org/10.1099/00207713-50-3-955

Dedysh SN, Derakshani M, Liesack W (2001) Detection and enumeration of methanotrophs in acidic sphagnum peat by 16S rRNA fluorescence in situ hybridization, including the use of newly developed oligonucleotide probes for *Methylocella palustris*. Appl Environ Microbiol 67:4850–4857. https://doi.org/10.1128/aem.67.10.4850-4857.2001

Dedysh SN, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Liesack W, Tiedje JM (2002) *Methylocapsa acidiphila* gen. nov., sp. nov., a novel methane-oxidizing and dinitrogen-fixing acidophilic bacterium from *Sphagnum* bog. Int J Syst Evol Microbiol 52:251–261. https://doi.org/10.1099/00207713-52-1-251

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. https://doi.org/10.1111/j.1574-6941.2003.tb01070.x

Dedysh SN, Berestovskaya YY, Vasylieva LV, Belova SE, Khmelenina VN, Suzina NE, Trotsenko YA, Liesack W, Zavarzin GA (2004) *Methylocella tundrae* sp. nov., a novel methanotrophic bacterium from acidic tundra peatlands. Int J Syst Evol Microbiol 54:151–156. https://doi.org/10.1099/ijs.0.02805-0

Dedysh SN, Knief C, Dunfield PF (2005) *Methylocella* species are facultatively methanotrophic. J Bacteriol 187:4665–4670. https://doi.org/10.1128/JB.187.13.4665-4670.2005

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. https://doi.org/10.1099/ijs.0.64623-0

Dedysh SN, Didriksen A, Danilova OV, Belova SE, Liebner S, Svenning MM (2015) *Methylocapsa palsarum* sp. nov., a methanotroph isolated from a sub-Arctic discontinuous permafrost ecosystem. Int J Syst Evol Microbiol 65:3618–3624. https://doi.org/10.1099/ijsem.0.000465

Dumont MG (2014) Primers: functional marker genes for methylotrophs and methanotrophs. In: McGenity TJ, Timmis KN, Nogales B (eds) Hydrocarbon and lipid microbiology protocols. Springer, Berlin, pp 57–77. https://doi.org/10.1007/8623_2014_23

Dunfield PF, Dedysh SN (2014) *Methylocella*: a gourmand among methanotrophs. Trends Microbiol 22:368–369. https://doi.org/10.1016/j.tim.2014.05.004.

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 53:1231–1239. https://doi.org/10.1099/ijs.0.02481-0

Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S, Ly B, Saw JH, Zhou Z, Ren Y (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum *Verrucomicrobia*. Nature 450:879–882. https://doi.org/10.1038/nature06411

Dunfield PF, Belova SE, Vorob'ev AV, Cornish SL, Dedysh SN (2010) *Methylocapsa aurea* sp. nov., a facultative methanotroph possessing a particulate methane monooxygenase, and emended description of the genus *Methylocapsa*. Int J Syst Evol Microbiol 60:2659–2664. https://doi.org/10.1099/ijs.0.020149-0

Erikstad H, Birkeland NK (2015) Draft genome sequence of "*Candidatus* Methylacidiphilum kamchatkense" strain Kam1, a thermoacidophilic methanotrophic *Verrucomicrobium*. Genome Announc 3:e00065. https://doi.org/10.1128/genomeA.00065-15

Esson KC, Lin X, Kumaresan D, Chanton JP, Murrell JC, Kostka JE (2016) Alpha- and gammaproteobacterial methanotrophs codominate the active methane-oxidizing communities in an acidic boreal peat bog. Appl Environ Microbiol 82:2363–2371. https://doi.org/10.1128/AEM.03640-15

Etiope G, Klusman R (2002) Geologic emissions of methane to the atmosphere. Chemosphere 49:777–789. https://doi.org/10.1016/S0045-6535(02)00380-6

Ettwig KF, Zhu B, Speth D, Keltjens JT, Jetten MSM, Kartal B (2016) Archaea catalyze iron-dependent anaerobic oxidation of methane. Proc Natl Acad Sci U S A 113:12792–12796. https://doi.org/10.1073/pnas.1609534113

Giggenbach W (1995) Variations in the chemical and isotopic composition of fluids discharged from the Taupo Volcanic Zone, New Zealand. J Volcanol Geotherm Res 68:89–116. https://doi.org/10.1016/0377-0273(95)00009-J

Gorham E (1991) Northern peatlands: role in the carbon cycle and probable response to climactic warming. Ecol Appl 1:183–195. https://doi.org/10.2307/1941811

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. https://doi.org/10.1111/j.1758-2229.2010.00237.x

Grodnitskaya ID, Trusova MY, Syrtsov SN, Koroban NV (2018) Structure of microbial communities of peat soils in two bogs in Siberian tundra and forest zones. Microbiology 87:89–102. https://doi.org/10.1134/s0026261718010083

Gupta V, Smemo KA, Yavitt JB, Basiliko N (2012) Active methanotrophs in two contrasting North American peatland ecosystems revealed using DNA-SIP. Microb Ecol 63:438–445. https://doi.org/10.1007/s00248-011-9902-z

Gupta V, Smemo KA, Yavitt JB, Fowle D, Branfireun B, Basiliko N (2013) Stable isotopes reveal widespread anaerobic methane oxidation across latitude and peatland type. Environ Sci Technol 47:8273–8279. https://doi.org/10.1021/es400484t

Hamilton R, Kits K, Ramonovskaya V, Rozova O, Yurimoto H, Iguchi H, Khmelenina V, Sakai Y, Dunfield PF, Klotz M, Knief C, Op den Camp HJM, Jetten MSM, Bringel F, Vuilleumier S, Svenning M, Shapiro N, Woyke T, Trotsenko YA, Stein L, Kaluzhnaya M (2015) Draft genomes of gammaproteobacterial methanotrophs isolated from terrestrial ecosystems. Genome Announc 3:e00515. https://doi.org/10.1128/genomeA.00515-15

Han D, Dedysh SN, Liesack W (2018) Unusual genomic traits suggest *Methylocystis bryophila* S285 to be well adapted for life in peatlands. Genome Biol Evol 10:623–628. https://doi.org/10.1093/gbe/evy025

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

Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P, Yuan Z, Tyson GW (2013) Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature 500:567–570. https://doi.org/10.1038/nature12375

Ho A, Kerckhof FM, Luke C, Reim A, Krause S, Boon N, Bodelier PL (2013) Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. Environ Microbiol Rep 5:335–345. https://doi.org/10.1111/j.1758-2229.2012.00370.x

Holmes AJ, Roslev R, 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

Hou S, Makarova KS, Saw JH, 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. https://doi.org/10.1186/1745-6150-3-26

Hribljan JA, Suárez E, Heckman KA, Lilleskov EA, Chimner RA (2016) Peatland carbon stocks and accumulation rates in the Ecuadorian páramo. Wetl Ecol Manag 24:113–127. https://doi.org/10.1007/s11273-016-9482-2

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 111:4495–4500. https://doi.org/10.1073/pnas.1318393111

Iguchi H, Yurimoto H, Sakai Y (2010) *Methylovulum miyakonense* gen. nov., sp. nov., a type I methanotroph isolated from forest soil. Int J Syst Evol Microbiol 61:810–815. https://doi.org/10.1099/ijs.0.019604-0

Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK (2008) Methane oxidation at 55°C and pH 2 by a thermoacidophilic bacterium belonging to the *Verrucomicrobia* phylum. Proc Natl Acad Sci U S A 105:300–304. https://doi.org/10.1073/pnas.0704162105

Islam T, Torsvik V, Larsen O, Bodrossy L, Ovreas L, Birkeland NK (2016) Acid-tolerant moderately thermophilic methanotrophs of the class *Gammaproteobacteria* isolated from tropical topsoil with methane seeps. Front Microbiol 7:851. https://doi.org/10.3389/fmicb.2016.00851

Jaatinen K, Tuittila ES, Laine J, Yrjälä K, Fritze H (2005) Methane-oxidizng bacteria in a Finnish raised mire complex: effects of site fertility and drainage. Microb Ecol 50:429–439. https://doi.org/10.1007/s00248-005-9219-x

Khadem AF, van Teeseling MC, van Niftrik L, Jetten MSM, Op den Camp HJM, Pol A (2012) Genomic and physiological analysis of carbon storage in the verrucomicrobial methanotroph "*Ca. Methylacidiphilum fumariolicum*" SolV. Front Microbiol 3:345. https://doi.org/10.3389/fmicb.2012.00345

Kip N, van Winden JM, Pan Y, Bodrossy L, Reichart GJ, Smolders AJP, Jetten MSM, Sinninghe Damsté JS, Op den Camp HJM (2010) Global prevalence of methane oxiation by symbiotic bacteria in peat-moss ecosystems. Nat Geosci 3:617–621. https://doi.org/10.1038/ngeo939

Kip N, Dutilh BE, Pan Y, Bodrossy L, Neveling K, Kwint MP, Jetten MSM, Op den Camp HJM (2011a) Ultra-deep pyrosequencing of *pmoA* amplicons confirms the prevalence of *Methylomonas* and *Methylocystis* in *Sphagnum* mosses from a Dutch peat bog. Environ Microbiol Rep 3:667–673. https://doi.org/10.1111/j.1758-2229.2011.00260.x

Kip N, Ouyang W, van Winden J, Raghoebarsing A, van Niftrik L, Pol A, Pan Y, Bodrossy L, van Donselaar EG, Reichart GJ, Jetten MSM, Damsté JS, Op den Camp HJM (2011b) Detection, isolation, and characterization of acidophilic methanotrophs from *Sphagnum* mosses. Appl Environ Microbiol 77:5643–5654. https://doi.org/10.1128/AEM.05017-11

Knief C (2015) Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on *pmoA* as molecular marker. Front Microbiol 6:1346. https://doi.org/10.3389/fmicb.2015.01346

Knief C, Dunfield PF (2005) Response and adaptation of different methanotrophic bacteria to low methane mixing ratios. Environ Microbiol 7:1307–1317. https://doi.org/10.1111/j.1462-2920.2005.00814.x

Knief C, Vanitchung S, Harvey NW, Conrad R, Dunfield PF, Chidthaisong A (2005) Diversity of methanotrophic bacteria in tropical upland soils under different land uses. Appl Environ Microbiol 71:3826–3831. https://doi.org/10.1128/AEM.71.7.3826-3831.2005

Knief C, Kolb S, Bodelier PL, Lipski A, Dunfield PF (2006) The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. Environ Microbiol 8:321–333. https://doi.org/10.1111/j.1462-2920.2005.00898.x

Kravchenko I, Kizilova A, Menko E, Sirin A (2015) Methane cycling microbial communities in natural and drained sites of Taldom Peatland, Moscow region, Russia. Ann Res Rev Biol 6:121–132. https://doi.org/10.9734/arrb/2015/14978

Krumholz LR, Hollenback JL, Roskes SJ, Ringelberg DB (1995) Methanogensis and methanotroph within a *Sphagnum* peatland. FEMS Microbiol Ecol 18:215–224. https://doi.org/10.1016/0168-6496(95)00061-4

Luesken FA, Wu ML, Op den Camp HJM, Keltjens JT, Stunnenberg H, Francoijs KJ, Strous M, Jetten MSM (2012) Effect of oxygen on the anaerobic methanotroph '*Candidatus Methylomirabilis oxyfera*': kinetic and transcriptional analysis. Environ Microbiol 14(4): 1024–1034. https://doi.org/10.1111/j.1462-2920.2011.02682.x

Martineau C, Whyte LG, Greer CW (2010) Stable isotope probing analysis of the diversity and activity of methanotrophic bacteria in soils from the Canadian high Arctic. Appl Environ Microbiol 76:5773–5784. https://doi.org/10.1128/AEM.03094-09

McDonald IR, Uchiyama H, Kambe S, Yagi K, Murrell JC (1997) The soluble methane monooxygenase gene cluster of the trichloroethylene-degrading methanotroph *Methylocystis* sp. strain M. Appl Environ Microbiol 63(5):1898–1904

Mohammadi S, Pol A, van Alen TA, Jetten MSM, Op den Camp HJM (2017) *Methylacidiphilum fumariolicum* SolV, a thermoacidophilic 'Knallgas' methanotroph with both an oxygen-sensitive and -insensitive hydrogenase. ISME J 11:945–958. https://doi.org/10.1038/ismej.2016.171

Morris SA, Radajewski S, Willison TW, Murrell JC (2002) Identification of the functionally active methanotroph population in a peat soil microcosm by stable-isotope probing. Appl Environ Microbiol 68:1446–1453. https://doi.org/10.1128/aem.68.3.1446-1453.2002

Omelchenko MV, Vasilieva LV, Zavarzin GA, Saveleva ND, Lysenko AM, Mityushina LL, Khmelenina VN, Trotsenko YA (1996) A novel psychrophilic methanotroph of the genus *Methylobacter*. Mikrobiologiya 65:384–389

Pagaling E, Yang K, Yan T (2014) Pyrosequencing reveals correlations between extremely acidophilic bacterial communities with hydrogen sulphide concentrations, pH and inert polymer coatings at concrete sewer crown surfaces. J Appl Microbiol 117:50–64. https://doi.org/10.1111/jam.12491

Page SE, Rieley JO, Banks CJ (2011) Global and regional importance of the tropical peatland carbon pool. Glob Chang Biol 17:798–818. https://doi.org/10.1111/j.1365-2486.2010.02279.x

Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MSM, Op den Camp HJM (2007) Methanotrophy below pH 1 by a new *Verrucomicrobia* species. Nature 450:874–878. https://doi.org/10.1038/nature06222

Pratscher J, Vollmers J, Wiegand S, Dumont MG, Kaster AK (2018) Unravelling the identity, metabolic potential and global biogeography of the atmospheric methane-oxidizing upland soil cluster α. Environ Microbiol. https://doi.org/10.1111/1462-2920.14036

Putkinen A, Larmola T, Tuomivirta T, Siljanen HM, Bodrossy L, Tuittila ES, Fritze H (2012) Water dispersal of methanotrophic bacteria maintains functional methane oxidation in *Sphagnum* mosses. Front Microbiol 3:15. https://doi.org/10.3389/fmicb.2012.00015

Putkinen A, Larmola T, Tuomivirta T, Siljanen HM, Bodrossy L, Tuittila ES, Fritze H (2014) Peatland succession induces a shift in the community composition of *Sphagnum*-associated active methanotrophs. FEMS Microbiol Ecol 88:596–611. https://doi.org/10.1111/1574-6941.12327

Raghoebarsing AA, Smolders AJP, Schmid MC, Rijpstra WIC, Wolters-Arts M, Derksen J, Jetten MSM, Schouten S, Sinninghe Damsté JS, Lamers LPM, Roelofs JGM, Op den Camp HJM, Strous M (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436:1153–1156. https://doi.org/10.1038/nature03802

Richter-Menge J, Mathis J (2017) The Arctic: overview. In: Blunden J, Arndt D (eds) State of the climate in 2016, vol 98. American Meteorological Society, Boston, p S129. https://doi.org/10.1175/2017BAMSStateoftheClimate.1

Ricke P, Kube M, Nakagawa S, Erkel C, Reinhardt R, Liesack W (2005) First genome data from uncultured upland soil cluster alpha methanotrophs provide further evidence for a close phylogenetic relationship to *Methylocapsa acidiphila* B2 and for high-affinity methanotrophy involving particulate methane monooxygenase. Appl Environ Microbiol 71:7472–7482. https://doi.org/10.1128/AEM.71.11.7472-7482.2005

Segers R (1998) Methane production and methane consumption: a review of processes underlying wetland methane fluxes. Biogeochemistry 41:23–51

Sharp CE, Op den Camp HJM, Tamas I, Dunfield PF (2013) Unusual members of the PVC superphylum: the methanotrophic *Verrucomicrobia* genus "*Methylacidiphilum*". In: Fuerst J (ed) *Planctomycetes*: cell structure, origins and biology. Springer, Berlin, pp 211–227

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 16:1867–1878. https://doi.org/10.1111/1462-2920.12454

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539. https://doi.org/10.1038/msb.2011.75

Siljanen HM, Saari A, Krause S, Lensu A, Abell GC, Bodrossy L, Bodelier PL, 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. https://doi.org/10.1111/j.1574-6941.2010.01015.x

Smemo KA, Yavitt JB (2007) Evidence for anaerobic CH_4 oxidation in freshwater peatlands. Geomicrobiol J 24:583–597. https://doi.org/10.1080/01490450701672083

Smemo KA, Yavitt JB (2011) Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems? Biogeosciences 8:779–793. https://doi.org/10.5194/bg-8-779-2011

Smith L, MacDonald G, Velichko A, Beilman D, Borisova O, Frey K, Kremenetski K, Sheng Y (2004) Siberian peatlands a net carbon sink and global methane source since the early Holocene. Science 303:353–355. https://doi.org/10.1126/science.1090553

Sundh I, Borga P, Nilsson M, Svensson BH (1995) Estimation of cell numbers of methanotrophic bacteria in boreal peatlands based on analysis of specific phospholipid fatty acids. FEMS Microbiol Ecol 18:103–112. https://doi.org/10.1111/j.1574-6941.1995.tb00167.x

Svenning MM, Hestnes AG, Wartiainen I, Stein L, Klotz MG, Kaluzhnaya M, Spang A, Bringel F, Vuilleumier S, Lajus A, Medigue C, Bruce D, Cheng J, Goodwin L, Ivanova N, Han J, Han C, Hauser LJ, Held B, Land M, Lapidus A, Lucas S, Nolan M, Pitluck S, Woyke T (2011) Genome sequence of the Arctic methanotroph *Methylobacter tundripaludum* SV96. J Bacteriol 193(22): 6418–6419. https://doi.org/10.1128/JB.05380-11

Tavormina PL, Orphan VJ, Kalyuzhnaya MG, Jetten MSM, Klotz MG (2011) A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. Environ Microbiol Rep 3:91–100. https://doi.org/10.1111/j.1758-2229.2010.00192.x

Tourova T, Omelchenko MV, Fegeding K, Vasilieva L (1999) The phylogenetic position of *Methylobacter psychrophilus* sp. nov. Mikrobiologiia 68:493–495

Trotsenko YA, Khmelenina VN (2005) Aerobic methanotrophic bacteria of cold ecosystems. FEMS Microbiol Ecol 53:15–26. https://doi.org/10.1016/j.femsec.2005.02.010

Tveit A, Schwacke R, Svenning MM, Urich T (2013) Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. ISME J 7:299–311. https://doi.org/10.1038/ismej.2012.99

Tveit AT, Ulrich T, Svenning MM (2014) Metatranscriptomic analysis of Arctic peat soil microbiota. Appl Environ Microbiol 80:5761–5772. https://doi.org/10.1128/AEM.01030-14

Tveit AT, Urich T, Frenzel P, Svenning MM (2015) Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming. Proc Natl Acad Sci U S A 112:E2507–E2516. https://doi.org/10.1073/pnas.1420797112

Vaksmaa A, van Alen TA, Ettwig KF, Lupotto E, Vale G, Jetten MSM, Luke C (2017) Stratification of diversity and activity of methanogenic and methanotrophic microorganisms in a nitrogen-fertilized Italian paddy soil. Front Microbiol 8:2127. https://doi.org/10.3389/fmicb.2017.02127

van Teesling MC, Pol A, Harhangi HR, van der Zwart S, Jetten MS, Op den Camp HJM, van Niftrik L (2014) Expanding the verrucomicrobial methanotrophic world: description of three novel species of *Methylacidimicrobium* gen. nov. Appl Environ Microbiol 80:6782–6781. https://doi.org/10.1128/AEM.01838-14

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 61:2456–2463. https://doi.org/10.1099/ijs.0.028118-0

Wartiainen I, Hestnes AG, McDonald IR, Svenning MM (2006) *Methylobacter tundripaludum* sp. nov., a methane-oxidizing bacterium from Arctic wetland soil on the Svalbard islands, Norway (78° N). Int J Syst Evol Microbiol 56:109–113. https://doi.org/10.1099/ijs.0.63728-0

Welte CU, Rasigraf O, Vaksmaa A, Versantvoort W, Arshad A, Op den Camp HJM, Jetten MSM, Luke C, Reimann J (2016) Nitrate- and nitrite-dependent anaerobic oxidation of methane. Environ Microbiol Rep 8:941–955. https://doi.org/10.1111/1758-2229.12487

Yu L, Huang Y, Zhang W, Li T, Sun W (2017) Methane uptake in global forest and grassland soils from 1981 to 2010. Sci Total Environ 607–608:1163–1172. https://doi.org/10.1016/j.scitotenv.2017.07.082

Yule CM, Lim YY, Lim TY (2016) Degradation of tropical Malaysian peatlands decreases levels of phenolics in soil and in leaves of *Macaranga pruinosa*. Front Earth Sci 4:45. https://doi.org/10.3389/feart.2016.00045

Zhu B, van Dijk G, Fritz C, Smolders AJM, Pol A, Jetten MSM, Ettwig KF (2012) Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent methane-oxidizing bacteria. Appl Environ Microbiol 78(24):8657–8665. https://doi.org/10.1128/AEM.02102-12

Microbial Communities in Hydrocarbon-Contaminated Desert Soils

9

Thirumahal Muthukrishnan and Raeid M. M. Abed

Contents

Abstract

Desert ecosystems are vulnerable to heavy crude oil spills during oil exploration and extraction processes. Oil-contaminated deserts exhibit harsh environmental conditions such as extreme temperatures, lack of water, nutrient deficiency, and the persistence of high concentrations of hydrocarbon compounds in soils. Over the past three decades, more attention has been directed to the study of oil-degrading microbial communities in oil-polluted desert ecosystems. In this chapter, current knowledge on hydrocarbon-degrading microbial communities in desert soils and their responses to bioremediation treatments as assessed using culture-based and molecular approaches has been reviewed. Diverse groups of

T. Muthukrishnan · R. M. M. Abed (✉)
College of Science, Biology Department, Sultan Qaboos University, Muscat, Sultanate of Oman
e-mail: mthirumahal@gmail.com; rabed@mpi-bremen.de

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_11

bacteria and fungi have been detected in oil-polluted desert soils despite the severe environmental conditions. Bioremediation approaches including landfarming, phytoremediation, and the use of nutrients and vitamin mixtures have proven to be successful in the cleanup of oil-polluted desert soils. However, bioaugmentation approaches have not succeeded in most cases due to the inability of exogenous microorganisms to compete with indigenous microorganisms in desert soils. Further investigations are required to scale up bioremediation treatments and test their applicability in field conditions. More research should also be focused on the use of genomic and proteomic approaches to study the functional diversity and activities of microorganisms in oil-polluted desert soils.

1 Introduction

Deserts are widely distributed in tropical, subtropical, temperate, and polar regions of the world (Delille et al. 2004a, b; Embar et al. 2006; Godoy-Faúndez et al. 2008; Saadoun et al. 2008; Reyes-Bozo et al. 2010; Al-Awadhi et al. 2012; Abed et al. 2015b; Ali et al. 2016a, b). These ecosystems exhibit harsh environmental conditions including extreme temperatures, lack of water, and nutrient deficiency (Noy-Meir 1973; Noble and Gitay 1996; Aislabie et al. 2006; Radwan 2008; Al-Kindi and Abed 2016a, b). Despite the severe physicochemical conditions, several groups of microorganisms including bacteria, fungi, actinomycetes, cyanobacteria, and microalgae survive in deserts due to their different adaptation mechanisms (Torsvik and Øvreås 2008; Radwan 2008). Desert microorganisms are also vulnerable to additional stress by crude oil pollution, mostly in the hot deserts of the oil-producing countries in the Arabian Peninsula. This has become evident following the massive crude oil spill after the Gulf War in 1991, where large areas of the deserts of Kuwait and Saudi Arabia were contaminated (Radwan 1990; McKinnon and Vine 1991; Radwan et al. 1995a, c). Large oil exploration facilities have been constructed in the Arabian Desert causing recurrent oil spills (McKinnon and Vine 1991; Radwan 2008; Abed et al. 2015b). Although oil spills have been frequently reported in deserts (McKinnon and Vine 1991; Aislabie et al. 2006; Saadoun et al. 2008; Radwan 2008, 2009; Sanscartier et al. 2009; Abed et al. 2015b; Radwan et al. 2017), only recently research has been performed to study the diversity of the oil-degrading microbial community structure in these ecosystems.

Various bioremediation treatments have been attempted to clean up oil-contaminated desert soils (Delille et al. 2004a, b; Radwan 2008; Dias et al. 2012; Abed et al. 2015b; Ali et al. 2016a, b; Álvarez et al. 2017). However, the extreme conditions of temperature, water availability, aeration, and nutrients hampered the success of bioremediation, not only by affecting the diversity of desert microorganisms but also by restricting their activities (Balba et al. 1998; Radwan 2008; Mahmoud et al. 2010; Sorkhoh et al. 2010a, b; Abed et al. 2015b; Al-Kindi and Abed 2016a, b; Ali et al. 2016a, b; Álvarez et al. 2017). Natural rates of biodegradation in deserts are expected to be very low (Sorkhoh et al. 1993;

Radwan et al. 1995c; Margesin and Schinner 2001; Radwan 2008; Abed et al. 2015b), which renders bioremediation of oil-contaminated desert soils very challenging. The applicability of bioremediation approaches like biostimulation and bioaugmentation to decontaminate desert soils has shown different degrees of success (Cho et al. 1997a, b; Delille et al. 2004a, b; Embar et al. 2006; Godoy-Faúndez et al. 2008; Al-Mailem et al. 2015; Abed et al. 2015a, b; Al-Kharusi et al. 2016a, b; Al-Kindi and Abed 2016a, b; Ali et al. 2016a, b; Álvarez et al. 2017; Radwan et al. 2017). The objective of this chapter is to present the current knowledge of indigenous hydrocarbon-degrading microbial communities in desert soils as reported using culture-dependent and culture-independent techniques and their responses to bioremediation treatments.

2 Cultivable Hydrocarbon-Degrading Desert Microorganisms

Hydrocarbon-degrading microorganisms have been isolated from hot and cold deserts (Kerry 1990; Whyte et al. 1997; Cho et al. 1997b; Sorkhoh et al. 1990, 1993, 1995, 2010a, b; Radwan 1998; Radwan et al. 1995b; Aislabie et al. 2000, 2001; Thomassin-Lacroix et al. 2001; Ruberto et al. 2005; Al-Awadhi et al. 2012; Al-Mailem et al. 2015; Ali et al. 2016a, b). Most of the hydrocarbon-degrading bacteria isolated from the hot deserts originated from the oil-contaminated desert soils of Kuwait (Sorkhoh et al. 1990, 1993, 1995, 2010a, b; Cho et al. 1997b; Radwan 1998; Radwan et al. 1995b; Mohamed et al. 2006; Dashti et al. 2008, 2015; Mahmoud et al. 2010; Al-Awadhi et al. 2012; Al-Mailem et al. 2015; Ali et al. 2016a, b), in addition to few reports from the Negev Desert (Embar et al. 2006) and part of the Syrian Desert (Saadoun et al. 2008). In cold deserts, most oil-degrading microbial isolation have been conducted on soil samples from Antarctica (Kerry 1990; Mac Cormack and Fraile 1997; Aislabie et al. 2000, 2001; Panicker et al. 2002; Eckford et al. 2002; Farrell et al. 2003; Ruberto et al. 2005), while few studies used samples from the Arctic region (Whyte et al. 1997; Thomassin-Lacroix et al. 2001).

2.1 Hot Deserts

Oil-degrading bacterial strains belonging to more than 40 genera have been isolated (Table 1) from different oil-contaminated desert soils in Kuwait (Sorkhoh et al. 1990, 1993, 1995, 2010a, b; Cho et al. 1997b; Radwan 1998; Radwan and Sorkhoh 1997; Radwan et al. 1995a, b; Mohamed et al. 2006; Dashti et al. 2008, 2009, 2015; Obuekwe et al. 2009; Mahmoud et al. 2010; Ali et al. 2012, 2016a, b; Al-Awadhi et al. 2012; Al-Mailem et al. 2015; Subhash et al. 2017). Most of these strains belonged to the genera *Micrococcus*, *Pseudomonas*, *Bacillus*, *Geobacillus*, *Arthrobacter*, and *Rhodococcus* (Sorkhoh et al. 1990, 1993, 1995; Cho et al. 1997b; Dashti et al. 2008; Al-Awadhi et al. 2012). All the strains were able to

Table 1 Hydrocarbon-degrading bacterial species isolated from oil-polluted desert soils of Kuwait and Jordan and their hydrocarbon growth substrates. Species level identification was done using 16S rRNA sequence analysis

Bacterial genera	Hydrocarbon growth substrate	References
Class: *Actinobacteria*		
Amycolatopsis	Crude oil	Al-Mailem et al. (2015)
Agrococcus	Aliphatic HC[a], aromatic HC[b]	Al-Awadhi et al. (2012)
Arthrobacter	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil, aliphatic HC	Sorkhoh et al. (1995)
Brevibacterium	Crude oil, *n*-octadecane, phenanthrene	Ali et al. (2012)
Cellulomonas	Aliphatic HC, aromatic HC	Ali et al. (2012)
Corynebacterium	Crude oil, *n*-octadecane, phenanthrene	Saadoun et al. (2008) and Ali et al. (2012)
Dietzia	Aliphatic HC, aromatic HC	Ali et al. (2012)
	Crude oil	Ali et al. (2016a)
Gordonia	Crude oil	Mahmoud et al. (2010)
Isoptericola	Crude oil	Al-Mailem et al. (2015)
Kocuria	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
Microbacterium	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b)
Micrococcus	Crude oil	Saadoun et al. (2008)
Micromonospora	Crude oil, *n*-octadecane, phenanthrene	Ali et al. (2012)
Mycobacterium	Aliphatic HC, aromatic HC	Ali et al. (2012)
	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b)
Nocardia	Crude oil	Al-Mailem et al. (2015)
	n-hexadecane	Cho et al. (1997b)
	Crude oil, aliphatic HC	Sorkhoh et al. (1990)
	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil, *n*-octadecane, phenanthrene	Ali et al. (2012)
	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b)
	Aliphatic HC, aromatic HC	Sorkhoh et al. (2010b)
	Crude oil	Ali et al. (2016a)
Rhodococcus	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil, aliphatic HC	Sorkhoh et al. (1990, 1995)
	n-hexadecane	Cho et al. (1997b)
	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b)
Streptomyces	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b)
	Crude oil, aliphatic HC	Sorkhoh et al. (1990, 1995)

(*continued*)

Table 1 (continued)

Bacterial genera	Hydrocarbon growth substrate	References
	Crude oil, *n*-octadecane, phenanthrene	Ali et al. (2012)
Williamsia	Crude oil	Obuekwe et al. (2009)
Class: *Bacilli*		
Aeribacillus	Crude oil	Al-Mailem et al. (2015)
Aneurinibacillus	Crude oil	Al-Mailem et al. (2015)
Anoxybacillus	Anthracene	Mohamed et al. (2006)
	Dibenzothiophene, benzothiophene	Mohamed et al. (2006)
Bacillus	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Anthracene, dibenzofuran	Mohamed et al. (2006)
	Phenanthrene	Mohamed et al. (2006)
	Phenanthrene	Mohamed et al. (2006)
	Crude oil, aliphatic HC	Sorkhoh et al. (1990, 1995) and Saadoun et al. (2008)
	Crude oil, *n*-octadecane, phenanthrene	Ali et al. (2012)
Brevibacillus	Crude oil	Al-Mailem et al. (2015)
Exiguobacterium	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010a)
Geobacillus	Crude oil	Al-Mailem et al. (2015)
	Crude oil, pentadecane, hexadecane, heptadecane	Sorkhoh et al. (1993)
	Phenanthrene	Mohamed et al. (2006)
Listeria	Crude oil	Saadoun et al. (2008)
Paenibacillus	Dibenzothiophene	Mohamed et al. (2006)
Saccharococcus	Dibenzothiophene	Mohamed et al. (2006)
Thermoactinomyces	Crude oil, aliphatic HC	Sorkhoh et al. (1995)
Class: *Alphaproteobacteria*		
Agrobacterium	Crude oil	Dashti et al. (2015)
Brevundimonas	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil	Ali et al. (2016a)
Chelativorans	Crude oil	Al-Mailem et al. (2015)
Skermanella	Crude oil	Subhash et al. (2017)
Roseomonas	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
Sphingobium	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
Sphingomonas	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil	Dashti et al. (2015)
Class: *Betaproteobacteria*		
Bordetella	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
Acidovorax	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b)
Alcaligenes	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b) and Ali et al. (2012)

(*continued*)

Table 1 (continued)

Bacterial genera	Hydrocarbon growth substrate	References
Chromobacterium	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010b)
Cupriavidus	Crude oil	Ali et al. (2016a)
Massilia	Crude oil	Ali et al. (2016a)
Oxalobacteraceae	Crude oil	Ali et al. (2016a)
Class: *Gammaproteobacteria*		
Acinetobacter	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil	Saadoun et al. (2008) and Dashti et al. (2015)
Enterobacter	Crude oil, *n*-octadecane, phenanthrene	Saadoun et al. (2008) and Ali et al. (2012)
Enterobacteriaceae	Crude oil, aliphatic HC	Sorkhoh et al. (1990)
Citrobacter	Crude oil, aliphatic HC	Sorkhoh et al. (2010a)
Marinobacter	Crude oil	Al-Mailem et al. (2015)
Pseudomonas	Aliphatic HC, aromatic HC	Al-Awadhi et al. (2012)
	Crude oil	Dashti et al. (2015) and Ali et al. (2016a)
	Crude oil, aliphatic HC	Sorkhoh et al. (1990, 1995)
	Crude oil, *n*-octadecane, phenanthrene	Sorkhoh et al. (2010a, b)
Stenotrophomonas	Crude oil	Saadoun et al. (2008)

[a]Aliphatic HC: *n*-alkanes ($C_{9–13}$, $C_{15–16}$, $C_{18–21}$, $C_{23–25}$, C_{28}, C_{30}, C_{36}, and C_{40})
[b]Aromatic HC: phenanthrene, naphthalene, and biphenyl

break down both aromatic and aliphatic hydrocarbons and their oxidation products, and some of them were thermophiles (Cho et al. 1997b; Dashti et al. 2008; Al-Awadhi et al. 2012). For instance, *Geobacillus stearothermophilus* was an obligate thermophilic strain with an optimum growth at 60 °C and an ability to consume crude oil as the sole source of carbon and energy (Sorkhoh et al. 1993). Some strains such as *Pseudomonas aeruginosa* KISR B1 and *Pseudomonas aeruginosa* KISR C1 produced biosurfactants that constituted different types of rhamnolipids (Yateem et al. 2002). Subsequent investigations were successful in isolating moderately thermophilic and hydrocarbonoclastic bacterial strains belonging to the genera *Amycolatopsis*, *Chelativorans*, *Isoptericola*, *Nocardia*, *Brevibacillus*, *Aeribacillus*, *Aneurinibacillus*, *Paenibacillus*, *Kocuria*, and *Marinobacter* (Al-Mailem et al. 2015).

Hydrocarbon-degrading bacterial strains have also been isolated from the rhizospheres of desert plants growing in the oil-contaminated Kuwaiti desert (Radwan et al. 1995b; Dashti et al. 2009; Mahmoud et al. 2010; Sorkhoh et al. 2010b). The most prevalent hydrocarbon-utilizing rhizospheric soil bacteria belonged to the genera *Cellulomonas*, *Rhodococcus*, and *Arthrobacter* (Radwan et al. 1995b). Consequent investigations reported the isolation of oil-utilizing diazotrophic rhizosphere bacteria belonging to the genera

Agrobacterium, *Arthrobacter*, *Gordonia*, *Bacillus*, *Ochrobactrum*, *Pseudoxanthomonas*, *Enterobacter*, *Micrococcus*, *Leifsonia*, *Rhizobium*, *Brevibacillus*, *Cellulosimicrobium*, *Stenotrophomonas*, *Kocuria*, *Pseudomonas*, *Microbacterium*, *Acidovorax*, *Mycobacterium*, *Rhodococcus*, *Alcaligenes*, and *Nocardia* (Dashti et al. 2009; Mahmoud et al. 2010; Sorkhoh et al. 2010b). On the other hand, nitrogen-fixing bacterial strains isolated from non-rhizospheric oily desert soil belonged to the genera *Pseudomonas*, *Exiguobacterium*, *Sphingomonas*, *Acinetobacter*, *Agrobacterium*, and *Dietzia* (Sorkhoh et al. 2010a; Al-Awadhi et al. 2012; Dashti et al. 2015). These strains could utilize aliphatic and aromatic hydrocarbons as sources of carbon and energy (Al-Awadhi et al. 2012; Dashti et al. 2015). Strains such as *Exiguobacterium aurantiacum* and *Pseudomonas putida* degraded crude oil despite the high concentrations (40 ppm) of mercuric chloride in the oil-polluted desert soil (Sorkhoh et al. 2010a). The abundance of such nitrogen-fixing bacteria in oil-contaminated desert soils was believed to compensate for the lack of nitrogen in these habitats (Radwan et al. 1995b; Sorkhoh et al. 2010b; Al-Awadhi et al. 2012; Dashti et al. 2015).

Oil-utilizing actinomycetes belonging to the genus *Streptomyces* were also isolated from oil-contaminated desert soils of Kuwait (Barabás et al. 1995, 2000; Radwan 1998; Al-Awadhi et al. 2012). Laboratory experiments demonstrated that selected strains of *Streptomyces* could degrade >90% of *n*-hexadecane within 4 months (Barabás et al. 2001). This was attributed to the ability of this microorganism to metabolize *n*-hexadecane to the fatty acid, *n*-hexadecanoic acid (Barabás et al. 2001). Isolates of hydrocarbon-degrading bacteria from other oil-contaminated hot deserts such as part of the Syrian Desert in Jordan (Table 1) were reported to belong to seven genera: *Stenotrophomonas*, *Acinetobacter*, *Enterobacter*, *Bacillus*, *Micrococcus*, *Corynebacterium*, and *Listeria* (Saadoun et al. 2008). The most dominant species was *Stenotrophomonas maltophilia* (Saadoun et al. 2008). Actinomycete strains from the same soil belonged to the genus *Streptomyces* (Saadoun et al. 2008). Furthermore, the study from the Negev Desert reported only the densities of isolated bacteria in the oil-polluted soils without revealing any information regarding the identity of the isolated bacterial strains (Embar et al. 2006).

Hydrocarbon-utilizing fungi belonging to seven genera have been isolated from the hot desert regions of Kuwait (Sorkhoh et al. 1990; Radwan et al. 2000), the Negev Desert (Embar et al. 2006), and part of the Syrian Desert in Jordan (Saadoun et al. 2008). Fungal strains belonging to the genera *Aspergillus* and *Penicillium* were the most dominant isolates from all oil-contaminated hot desert regions (Sorkhoh et al. 1990; Radwan et al. 2000; Embar et al. 2006; Saadoun et al. 2008). Strains belonging to the genera *Fusarium*, *Mucor*, *Microsporum*, and *Ulocladium* were isolated only from Kuwaiti desert soil (Sorkhoh et al. 1990; Radwan et al. 2000). All the fungal strains degraded medium-chain *n*-alkanes and few aromatic hydrocarbons, and the most active hydrocarbon-degrading fungus was the strain *Mucor globosus* (Sorkhoh et al. 1990). The study from the Negev Desert reported the dominance of the fungus

Memnoniella sp. that was capable of degrading 61% of the crude oil within 24 days (Embar et al. 2006).

2.2 Cold Deserts

Hydrocarbon-degrading bacteria and fungi have also been isolated from oil-contaminated cold desert soils (Kerry 1990; Whyte et al. 1997; Mac Cormack and Fraile 1997; Bej et al. 2000; Aislabie et al. 2000, 2001; Thomassin-Lacroix et al. 2001; Eriksson et al. 2002; Eckford et al. 2002; Farrell et al. 2003; Ruberto et al. 2005; Saul et al. 2005; Hughes et al. 2007). Hydrocarbon-degrading bacteria isolated from the Arctic and Antarctic desert soils belonged mainly to *Rhodococcus*, *Pseudomonas*, and *Sphingomonas* (Table 2) (Kerry 1990; Whyte et al. 1997; Mac Cormack and Fraile 1997; Master and Mohn 1998; Yu et al. 2000; Bej et al. 2000; Aislabie et al. 2000; Thomassin-Lacroix et al. 2001; Panicker et al. 2002; Eriksson et al. 2002; Baraniecki et al. 2002; Eckford et al. 2002; Farrell et al. 2003; Ruberto et al. 2005; Saul et al. 2005). The bacterial isolates affiliated to *Rhodococcus* were able to consume a wide range of *n*-alkanes (Bej et al. 2000), mainly due to the prevalence of alkane degradation gene Rh *alkB1* (Whyte et al. 2002a, b). The *alkB* gene, encoding for alkane monooxygenase, was one of the components of the alkane hydroxylase system that mediated alkane degradation processes in polar desert soils (Aislabie et al. 2006). Some species of *Rhodococcus* were also able to secrete biosurfactants such as trehalose mycolate lipids that enhanced the solubility and subsequent degradation of solid alkanes at low temperatures (Whyte et al. 1999). Bacterial species belonging to *Rhodococcus* could not degrade aromatic hydrocarbons (Bej et al. 2000), unlike species belonging to *Pseudomonas* and *Sphingomonas* (Whyte et al. 1997; Aislabie et al. 2000; Eckford et al. 2002; Farrell et al. 2003). Some *Pseudomonas* isolates exhibited nitrogen-fixing activity in situ while utilizing aromatic compounds for their growth (Eckford et al. 2002). *Sphingomonas* spp. were reported to utilize several aromatic hydrocarbons, heterocyclic and substituted hydrocarbon compounds, aromatic acids and alcohols (Baraniecki et al. 2002). Certain aromatic hydrocarbon substrates, such as biphenyl, triphenylene, dibenzofuran, anthracene, 2-methylanthracene, fluoranthene, pyrene, chrysene, dibenzothiophene, and 2-phenylnaphthalene, could not be utilized by these strains for their growth but were transformed or solubilized in the culture medium (Baraniecki et al. 2002).

Very little is known about hydrocarbon-utilizing fungi in cold desert regions (Kerry 1990; Aislabie et al. 2001; Hughes et al. 2007; Hassan et al. 2016). The significant increase in fungal abundance in different oil-contaminated sites in Antarctica indicated the potential role of fungi in hydrocarbon degradation (Kerry 1990; Aislabie et al. 2001; Hughes et al. 2007). Eight genera, including *Phialophora*, *Mortierella*, *Mucor*, *Penicillium*, *Phoma*, *Trichoderma*, *Mollisia*, and *Cladosporium*, were isolated (Table 2), with the genera *Phialophora* and *Mollisia* as the most dominant (Kerry 1990; Aislabie et al. 2001; Hughes et al.

Table 2 Hydrocarbon-degrading microorganisms isolated from oil-polluted polar desert soils of Arctic and Antarctic regions and their hydrocarbon growth substrates

Class	Genus	Growth substrate	References
Bacteria			
Alphaproteobacteria	*Sphingomonas*	Crude oil, phenanthrene, fluorene, heptane, undecane, dodecane, pristane, 1-methyl naphthalene, 2-methyl naphthalene, 2-ethyl naphthalene, *m*-xylene, 1,2,4-trimethyl benzene	Yu et al. (2000), Aislabie et al. (2000, 2006), Baraniecki et al. (2002), and Saul et al. (2005)
Gammaproteobacteria	*Pseudomonas*	Crude oil, biphenyl, naphthalene, 1-methyl naphthalene, 2-methyl naphthalene, toluene, pyrene, *m*-xylene, *p*-xylene, 1,2,4-trimethyl benzene, benzene, $C_{5–12}$ alkanes	Whyte et al. (1997), Master and Mohn (1998), Yu et al. (2000), Aislabie et al. (2000, 2006), Thomassin-Lacroix et al. (2001), Eckford et al. (2002), Eriksson et al. (2002), Panicker et al. (2002), and Farrell et al. (2003)
	Acinetobacter	Crude oil, dodecane, hexadecane, cyclohexane	Mac Cormack and Fraile (1997)
Actinobacteria	*Rhodococcus*	Crude oil, $C_{6–20}$ alkanes, pristane	Whyte et al. (1997), Bej et al. (2000), Saul et al. (2005), Ruberto et al. (2005), and Aislabie et al. (2006)
	Arthrobacter	Hexadecane	Pruthi and Cameotra (1997)
Fungi			
Eurotiomycetes	*Phialophora*	Pyrene	Ravelet et al. (2000)
	Penicillium	Crude oil, hydroxybenzoic acid	Kerry (1990) and Hughes et al. (2007)
Mucoromycotina	*Mortierella*	Crude oil, dodecane, hexadecane	Kerry (1990) and Hughes et al. (2007)
Mucoromycotina	*Mucor*	Crude oil	Kerry (1990)
Dothideomycetes	*Cladosporium*	Crude oil	Kerry (1990)
	Phoma	Dodecane, hexadecane	Hughes et al. (2007)
Ascomycetes	*Mollisia*	Dodecane, hexadecane	Hughes et al. (2007)
Sordariomycetes	*Trichoderma*	Dodecane, hexadecane	Hughes et al. (2007)

2007). *Phialophora* spp. were reported to degrade pyrene (Ravelet et al. 2000), whereas species belonging to *Mollisia*, *Trichoderma*, *Phoma*, and *Mortierella* could utilize dodecane and hexadecane as the sole carbon source for growth (Hughes et al. 2007).

3 Oil-Degrading Desert Microorganisms Revealed by Molecular Techniques

3.1 Hot Deserts

Most of the studies using culture-independent techniques to describe microbial diversity in oil-polluted desert soils have been done in the hot deserts of Kuwait and Oman (Al-Mailem et al. 2015; Abed et al. 2015a, b; Al-Kharusi et al. 2016a, b; Al-Kindi and Abed 2016a, b). Denaturing gradient gel electrophoresis (DGGE) and 16S rRNA sequencing techniques detected sequences affiliated to seven bacterial classes in the oil-contaminated desert soils in Kuwait (Al-Mailem et al. 2015). The *Gammaproteobacteria* constituted 20% of total sequences, while the classes *Alphaproteobacteria*, *Bacilli*, *Clostridia*, *Deltaproteobacteria*, *Actinobacteria*, and *Ignavibacteria* constituted $\leq$10% each (Al-Mailem et al. 2015). Many of these bacteria belonged to known hydrocarbonoclastic species, while others were affiliated to sulfur-reducing bacteria (Al-Mailem et al. 2015). Another fingerprinting technique called automated rRNA intergenic spacer analysis (ARISA) was used to study the differences in bacterial diversity along a petroleum pollution gradient in oil-polluted desert soils from Oman (Abed et al. 2015a). The ARISA profiles revealed that <20% of the operational taxonomic units (OTUs) were shared among the different soils and the OTU richness was relatively low in the soil exposed to the lowest oil contamination (Abed et al. 2015a). Using 454 pyrosequencing on the same samples, it was demonstrated that the soils with the highest oil contamination level were dominated by *Deltaproteobacteria* (30% of total sequences) and *Clostridia* (13%). *Deltaproteobacteria* included mostly sulfate-reducing bacterial genera such as *Desulfococcus*, *Desulfosalsimonas*, and *Desulfovermiculus*, while most of the *Clostridia* sequences belonged to the genus *Halanaerobium.* In the soils with the lowest oil contamination, *Acidobacteria* (<30% of total sequences), *Betaproteobacteria* (<30%), *Sphingobacteria* (<10%), and *Deinococci* (<10%) were detected. Most sequences of *Acidobacteria* belonged to the genus *Terriglobus* (24% of total sequences). This study suggested that high oil concentrations in desert soils favored the growth of anaerobic bacteria such as the sulfate-reducing genera and *Clostridia*-related genera, while minimal oil concentrations allowed the non-hydrocarbon-degrading acidobacterial genus *Terriglobus* to outcompete hydrocarbon-degrading bacteria (Abed et al. 2015a).

MiSeq sequencing was used to investigate the bacterial community structure in oil-contaminated desert soils from different locations of Oman (Abed et al. 2015b; Al-Kharusi et al. 2016a, b; Al-Kindi and Abed 2016a, b). Bacteria belonging to *Alphaproteobacteria* and *Gammaproteobacteria* were detected in all studied desert soils, regardless of their location. However, in oil-polluted desert soils adjacent to a sludge farm, bacteria belonging to *Bacilli*, *Betaproteobacteria*, and *Actinobacteria* were also detected.

Most dominant *Bacilli*-related genera included *Bacillus*, *Paenibacillus*, and *Planomicrobium* (Abed et al. 2015b). Desert soils collected near an oil production facility revealed the dominance of *Deinococci* bacteria (Al-Kharusi et al. 2016a, b), while oil-polluted soils collected from a dumping area in the desert revealed the prevalence of *Firmicutes* bacteria (Al-Kindi and Abed 2016a, b). Relatively high abundance of *Deinococci* in the desert soils highlighted the presence of potential UV and radiation-resistant hydrocarbon-degrading bacteria in hot deserts. Bacterial genera belonging to *Firmicutes* were also previously detected in fuel-contaminated soils (Sutton et al. 2013; Yang et al. 2014). This bacterial group included hydrocarbon-degrading genera such as *Bacillus* that could produce biosurfactants (Makut and Ishaya 2010; Sutton et al. 2013; Yang et al. 2014; Abed et al. 2015a).

3.2 Cold Deserts

Terminal restriction fragment length polymorphism (T-RFLP) was used to study microbial communities in the oil-contaminated desert soils in Chile; however no information on the identity of oil-degrading microbial communities was reported (Reyes-Bozo et al. 2010). Later investigations in the desert soils of the Arctic region used 454 pyrosequencing of 16S rRNA genes and quantitative real-time PCR (qPCR) for studying the prevalence of *alkB* genes (Bell et al. 2011). The detected 16S rRNA and *alkB* sequences were affiliated to *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Actinobacteria*, with the latter two classes as the most dominant (Bell et al. 2011). The most dominant genera belonging to *Gammaproteobacteria* were *Pseudomonas*, *Pseudoxanthomonas*, *Azomonas*, *Thermomonas*, *Lysobacter*, *Nevskia*, and *Steroidobacter*. In case of *Actinobacteria*, the most prevalent genera were *Rhodococcus*, *Agrococcus*, *Salinibacterium*, *Pimelobacter*, *Aeromicrobium*, and *Nocardioides* (Bell et al. 2011). The genera *Rhodococcus* and *Pseudomonas* were well-known for their hydrocarbon-degrading ability in the cold oil-contaminated desert soils, not only of Arctic region but also of Antarctica (Whyte et al. 1999, 2002a, b; Bej et al. 2000; Eckford et al. 2002; Farrell et al. 2003; Ruberto et al. 2005; Aislabie et al. 2000, 2006; Yergeau et al. 2012). Other studies using molecular tools in the oil-contaminated soils of the Arctic region also reported the dominance of bacteria belonging to *Gammaproteobacteria*, *Actinobacteria*, *Betaproteobacteria*, and *Chloroflexi* (Akbari and Ghoshal 2015). While the genus *Thermomonas* dominated the class *Gammaproteobacteria*, the prevalent genera detected in *Actinobacteria* included *Dietzia*, *Agromyces*, *Salinibacterium*, *Arthrobacter*, *Mycobacterium*, *Rhodococcus*, and *Williamsia* (Akbari and Ghoshal 2015). *Thermomonas* spp. were capable of degrading weathered hydrocarbons (high molecular weight) in sandy soils (Kaplan and Kitts 2004), while *Williamsia* spp. were able to utilize nitroaromatic compounds (Obuekwe et al. 2009; Thompson et al. 2005).

4 Bioremediation Treatments and Desert Microorganisms

Since the past two decades, bioremediation treatments including landfarming (Balba et al. 1998; El-Nawawy et al. 1999; Yateem et al. 2000; Al-Mahruki et al. 2006), phytoremediation (El-Nawawy et al. 1999; Yateem et al. 2000; Mahmoud et al. 2010; Sorkhoh et al. 2010b; Yateem 2013), biostimulation, and bioaugmentation (Cho et al. 1997a, b; Delille et al. 2004a, b; Embar et al. 2006; Al-Mueini et al. 2007; Godoy-Faúndez et al. 2008; Al-Mailem et al. 2015; Abed et al. 2015a, b; Al-Kharusi et al. 2016a, b; Al-Kindi and Abed 2016a, b; Ali et al. 2016a, b; Álvarez et al. 2017; Radwan et al. 2017) have been attempted to clean up oil-contaminated soils from hot and cold deserts. The impact of these bioremediation treatments on desert microbial communities was assessed using both culture-dependent (Cho et al. 1997a, b; Balba et al. 1998; El-Nawawy et al. 1999; Yateem et al. 2000; Thomassin-Lacroix et al. 2002; Delille et al. 2004a, b; Embar et al. 2006; Godoy-Faúndez et al. 2008; Mahmoud et al. 2010; Sorkhoh et al. 2010b; Yateem 2013; Al-Mailem et al. 2015; Ali et al. 2016a, b) and culture-independent techniques (Dashti et al. 2009; Akbari and Ghoshal 2015; Abed et al. 2015a, b; Al-Kharusi et al. 2016a, b; Al-Kindi and Abed 2016a, b).

4.1 Landfarming and Phytoremediation

Landfarming treatment of oil-polluted desert soils from Kuwait resulted in the gradual increase of bacterial and fungal abundances over a period of 6 months (Balba et al. 1998). This increase was associated with 85% reduction in the concentration of total alkanes (Balba et al. 1998). Landfarming was also used as a pretreatment process to facilitate the phytoremediation of oily desert soil in Kuwait (El-Nawawy et al. 1999; Yateem et al. 2000). Prior to phytoremediation, oil-polluted desert soil was subjected to 18 months of landfarming treatment, followed by cultivation of legume crops for 7 months. This treatment resulted in an increase in the total abundance of culturable hydrocarbon-degrading bacteria in the rhizospheric soil (El-Nawawy et al. 1999; Yateem et al. 2000) concomitant with a 36–37% reduction in the total petroleum hydrocarbon (TPH) content (Yateem et al. 2000) and 74% reduction in the residual polycyclic aromatic hydrocarbons (PAHs) (El-Nawawy et al. 1999).

When oil-polluted desert soils were used for cultivating desert ornamental plants such as Bermuda grass and American grass, the total numbers of oil-degrading cultivable bacteria increased significantly in the oil-polluted rhizospheric soil (Mahmoud et al. 2010). This occurred mainly due to a 39% increase in the total number of bacteria belonging to the genus *Gordonia*. The addition of crude oil to pristine desert soil with plant cover also inhibited the growth of the fungus *Fusarium* (Mahmoud et al. 2010). Similar findings pertaining to differences in total bacterial abundance in oily and non-oily desert soils were reported in phytoremediation treatments using both legume and non-legume crops (Sorkhoh et al. 2010b). The dominant bacteria isolated from the pristine rhizospheric soil belonged to the genera

Microbacterium (50% of total colony-forming unit or CFU) and *Pseudomonas* (26–30% of total CFU). When both legume and non-legume crops were grown in oily desert soil, the abundance of *Microbacterium* decreased (37–40% of total CFU) while that of *Pseudomonas* increased (37–46% of total CFU) (Sorkhoh et al. 2010b). Furthermore, higher bacterial abundance was reported in the rhizospheric soil of legume crops than that in non-legume crops. This seems to be associated with nitrogen fixation potential by the roots of legume crops that can eventually favor the growth of hydrocarbon-degrading bacteria (Leahy and Colwell 1990).

4.2 Biostimulation

Biostimulation efforts undertaken in the hot deserts used various methods to enhance oil biodegradation processes (Radwan and Al-Muteirie 2001; Embar et al. 2006; Al-Mailem et al. 2015; Abed et al. 2015b; Al-Kindi and Abed 2016a, b). Preliminary investigations used a mixture of vitamin B12, thiamine, pyridoxine, and folic acid to fertilize oil-contaminated desert soils in Kuwait for a period of 4 weeks (Radwan and Al-Muteirie 2001). Prevalent bacterial strains isolated from the oily desert soil prior to incubation belonged to *Cellulomonas*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, and *Arthrobacter* with the dominance of the bacterial strains *Cellulomonas flavigena* and *Rhodococcus erythropolis* (Radwan and Al-Muteirie 2001). Following the incubation of soils with the vitamin mixture, the total number of bacteria belonging to the strain *Cellulomonas flavigena* increased by 22%, while those belonging to the strain *Rhodococcus erthyropolis* decreased by 18% (Radwan and Al-Muteirie 2001). The vitamin-fertilized oily desert soil showed 42% reduction in the total alkane content compared to only 14% in the vitamin-free soils (Radwan and Al-Muteirie 2001). Another study used the soil bulking agent vermiculite (50% v/v) to biostimulate oil-polluted desert soils (Embar et al. 2006). While the abundance of bacteria in the treated soils rapidly decreased during the initial days of the treatment and then increased subsequently, the high abundance of fungi persisted throughout the treatment (Embar et al. 2006). Concurrently, maximal biodegradation rates (91%) were also obtained in the soil, indicating that the indigenous hydrocarbon-degrading fungal population required soil aeration to consume crude oil (Embar et al. 2006).

Biostimulation by moistening and continuous incubation at 50 °C or a period of 6 months was applied on desert soils from Kuwait collected during the winter and summer seasons (Al-Mailem et al. 2015). The total number of culturable bacteria increased significantly every month in both seasonal samples and reached a total of 10^3 cells g^{-1} at the end of the incubation. DGGE profiles revealed major differences in the soil after treatment depending on the location and season. The bacterial species *Melioribacter roseus* was frequently detected in the summer samples of all locations, while five other bacterial species were identified in the summer samples of two locations. The presence of sulfur-reducing bacteria and unculturable haloarchaea was also detected in two out of three locations (Al-Mailem et al. 2015). Later biostimulation treatments in the Kuwaiti desert soils involved the stimulation of

indigenous oil-utilizing bacterial isolates rather than enhancing the biodegradation capacity of the total bacterial community. A total of 19 species of indigenous, thermophilic, hydrocarbon-degrading bacteria isolated from oil-polluted desert soil of Kuwait were stimulated using Ca^{2+} and dipicolinic acid (DPA) (Radwan et al. 2017). Most of these isolates showed enhanced resistance to high concentrations of heavy metals as well as higher oil consumption rates in the presence of heavy metals (Radwan et al. 2017). Chemical complex of DPA and Ca^{2+} was previously reported to provide stability to nucleic acids and enzymes within microbial cells exposed to elevated temperatures, thereby stimulating microbial activity in hot desert soils (Gerhardt and Marquis 1989; Setlow et al. 2006; Radwan et al. 2017).

Pyrosequencing was used to investigate effects of different temperatures and salinities on the bacterial community structure in nutrient-amended oil-polluted desert soils from Oman (Abed et al. 2015b). In the soils incubated at 10 °C, *Gammaproteobacteria* (91% of the total sequences) and *Bacilli* (5% of total sequences) were the most prevalent classes of bacteria. At 30 °C, the relative abundances of *Gammaproteobacteria* and *Bacilli* shifted to 15% and 44% of the total sequences, respectively. Other classes such as *Alphaproteobacteria*, *Betaproteobacteria*, and *Clostridia* were also detected. Incubation of the oil-polluted soil at 50 °C resulted in the detection of the same bacterial classes but with different genera than those observed at 30 °C (Abed et al. 2015b). In case of biostimulated oily desert soils at different salinities, remarkable changes were revealed in the bacterial community structure. At 2% salinity, the classes *Gammaproteobacteria*, *Flavobacteria*, *Bacilli*, *Alphaproteobacteria*, and *Clostridia* constituted 96% of the total sequences. At 4% salinity, the relative abundance of *Gammaproteobacteria* decreased by 35% while that of *Actinobacteria* increased by 21% (Abed et al. 2015b). At 7% salinity, profound shifts in the relative abundance of *Gammaproteobacteria* (75% of total sequences) were observed.

Illumina MiSeq sequencing was used to follow shifts in microbial communities following biostimulation of oil-contaminated desert soils from Oman using organic wastes such as spent mushroom compost (SMC), urea, poultry manure (PM), sewage sludge (SG), soybean meal (SB), and wheat straw (WS) (Al-Kindi and Abed 2016a, b). The relative abundance of most bacterial groups did not differ much between treatments but that of *Firmicutes* showed treatment-specific changes (Al-Kindi and Abed 2016a, b). Bacteria belonging to *Firmicutes* constituted 37–48% of total sequences in the PM treatment of oil-contaminated soil (Al-Kindi and Abed 2016a). However, the relative abundance of this group increased to 44–87% of total sequences in the urea treatment but decreased to <5% of total sequences in the SMC treatment. In case of SB treatment, *Firmicutes* constituted >50% of total sequences (Al-Kindi and Abed 2016b). The WS treatment induced the most prominent changes in the total bacterial community in the soils leading to a community shift in favor of *Firmicutes* (95–98% of total sequences). The dominant genera in this treatment included *Bacillus*, *Virgibacillus*, *Salinibacillus*, and *Salirhabdus*. The detection of the aerobic *Bacilli*-related species indicates aerobic conditions in the soil, and this can be due to the role of WS as a bioventing agent to increase aeration in soil (Al-Kindi and Abed 2016b).

Biostimulation of oil-contaminated desert soils of Antarctica using fertilizers showed that the abundance of hydrocarbon-degrading bacteria increased considerably after treatment (Delille et al. 2004a, b; Álvarez et al. 2017). This was correlated to nearly complete degradation of aliphatic hydrocarbons after 1 year (Delille et al. 2004a). The total microbial activity also increased by more than ninefold after 30 days (Álvarez et al. 2017). Bioremediation of fuel-contaminated soils from Atacama Desert involved the addition of sawdust but revealed no significant changes in the total counts of culturable bacteria (Godoy-Faúndez et al. 2008).

4.3 Bioaugmentation

The introduction of laboratory-grown oil-degrading microorganisms to contaminated sites in the form of single or mixed cultures to speed up bioremediation processes in desert soils is very challenging. This is due to the inability of introduced strains to survive the harsh desert conditions and to compete with indigenous microorganisms. Bioaugmentation treatments of the oil-contaminated Kuwait desert soils using imported *Arthrobacter* strains revealed not only the failure of these strains to colonize the soil but also resulted in a rapid decline in the abundance of indigenous microorganisms (Radwan and Sorkhoh 1997). Further bioaugmentation efforts were based on the concept of using indigenous microorganisms in the form of naturally existing mixed communities rather than pure cultures or microbial consortium (Ali et al. 2016a, b). For instance, freshly oil-contaminated desert soils from Kuwait were bioremediated by mixing up with either coastal microbial mats (Ali et al. 2016a) or pea rhizosphere suspension or long-term oil-polluted desert soil (Ali et al. 2016b). The total number of culturable hydrocarbon-degrading bacteria in all treatments showed a gradual monthly increase until 6 months (Ali et al. 2016a, b). In the coastal microbial mat treatment, the indigenous hydrocarbon-degrading bacteria from the microbial mats successfully colonized the desert soil. Both the indigenous mat bacterium *Xanthobacter tagetidis* and the indigenous soil bacterium *Pseudomonas pachastrellae* persisted throughout the experiment (Ali et al. 2016a). All the isolates from the treated soil showed the ability to degrade 17–32% of crude oil. In the bioaugmentation treatment using pea rhizosphere suspension, the most dominant colonizers throughout the treatment included the strains *Arthrobacter nitroguajacolicus*, *Caulobacter segnis*, and *Ensifer adhaerens* (Ali et al. 2016b). Treatments using long-term oil-polluted desert soil revealed the dominance of only the two strains *Pseudomonas stutzeri* and *Massilia timonae*. The initial concentration of the crude oil used in all treatments was 1% w/v, and overall oil consumption values reached up to 98% within 4 months of treatments. Fresh samples of the desert soil bioaugmented with pea rhizosphere or long-term oil-polluted soil showed significantly higher oil consumption rates in comparison to autoclaved desert soil (Ali et al. 2016b). This indicates that indigenous microorganisms in one habitat can coexist with those from other habitats and simultaneously enhance bioremediation processes in oil-contaminated desert soils.

Oil-contaminated soils in hot deserts were also subjected to a combination of bioaugmentation and biostimulation treatments, and the treatment-induced effects on the microbial communities were investigated (Cho et al. 1997a, b; Al-Kharusi et al. 2016a, b). When oil-contaminated desert soils from Kuwait were biostimulated using soil conditioning agents such as bark manure, Hyponex powder, and baked diatomite for a period of 8 months, the most dominant bacterial strains isolated belonged to *Rhodococcus* (Cho et al. 1997a, b). However, biostimulation of the same oil-contaminated soils combined with bioaugmentation using oil-decomposing bacterial consortium (ten strains) for 8 months resulted in the dominance of *Nocardia* sp. (Cho et al. 1997a, b). Studies conducted in the oil-polluted desert soils from Oman used MiSeq to investigate the impact of combined biostimulation and bioaugmentation treatments on indigenous soil bacterial communities (Al-Kharusi et al. 2016a, b). Biostimulation treatments included the addition of quorum-sensing signals (acyl homoserine lactone or AHL) or chelating agent (ethylenediaminetetraacetic acid or EDTA) (Al-Kharusi et al. 2016a, b). Bioaugmentation treatment included the inoculation of an alkane-degrading bacterial consortium (Abed et al. 2014). The two inoculated strains *Alcanivorax* sp. and *Parvibaculum* sp. from the consortium persisted until the end of the treatments in both studies (Al-Kharusi et al. 2016a, b). The concentrations of total alkanes (C_{14}–C_{30}) in the bioaugmented soils treated with AHLs and EDTA (10mM) revealed 93% and 91% reductions, respectively. Concurrent increases in the bacterial respiration activities were observed in both studies for all bioaugmented and non-bioaugmented soils. However, MiSeq analyses of the total bacterial community structure revealed that the classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Deinococci*, and *Bacilli* were the most prevalent bacterial classes in the studied soils (Al-Kharusi et al. 2016a, b). The most remarkable changes were observed in the treatment using both EDTA and consortium. The relative abundances of *Flavobacteria* and *Bacilli* increased to 2–13% and 3–10% of the total sequences, respectively, while that of *Deinococci* reduced to 27–45% of total sequences (Al-Kharusi et al. 2016b). MiSeq provided adequate information regarding the fate of the inoculated bacterial consortium (bioaugmentation) and the knowledge required to select for highly competitive bacterial strains from oil-contaminated desert soils for future bioremediation treatments.

5 Research Needs

Oil-contaminated soils in the hot and cold desert ecosystems harbor various hydrocarbon-degrading bacteria and fungi. However, the role of archaea and anaerobic microorganisms in hydrocarbon degradation of desert soils is still unknown. Prospective research should focus on understanding the activity or function of hydrocarbon-degrading microorganisms in desert soils using functional genomic or proteomic approaches. This will provide a deeper insight into the biochemical mechanisms underlying hydrocarbon degradation processes mediated by desert microorganisms. Further studies are also required for the isolation of novel

oil-degrading extremophiles and to investigate their potential biotechnological applications. Future bioremediation attempts in oil-contaminated desert soils should be designed at a larger scale, and more attention needs to be given to testing their applicability in the field.

References

Abed RMM, Al Sabahi J, Al Maqrashi F, Al Habsi A, Al Hinai M (2014) Characterization of hydrocarbon degrading bacteria isolated from oil contaminated sediment in the Sultanate of Oman and evaluation of bioaugmentation and biostimulation approaches in microcosm experiments. Int Biodeterior Biodegrad 89:58–66

Abed RMM, Al-Kindi S, Al-Kharusi S (2015a) Diversity of bacterial communities along a petroleum contamination gradient in desert soils. Microb Ecol 69:95–105

Abed RMM, Al-Kharusi S, Al-Hinai M (2015b) Effect of biostimulation, temperature and salinity on respiration activities and bacterial community composition in an oil polluted desert soil. Int Biodeterior Biodegrad 98:43–52

Aislabie J, Foght J, Saul D (2000) Aromatic hydrocarbon-degrading bacteria from soil near Scott Base, Antarctica. Polar Biol 23:183–188

Aislabie J, Fraser R, Duncan S, Farrell RL (2001) Effects of oil spills on microbial heterotrophs in Antarctic soils. Polar Biol 24:308–313

Aislabie J, Saul DJ, Foght JM (2006) Bioremediation of hydrocarbon-contaminated polar soils. Extremophiles 10:171–179

Akbari A, Ghoshal S (2015) Effects of diurnal temperature variation on microbial community and petroleum hydrocarbon biodegradation in contaminated soils from a sub-Arctic site. Environ Microbiol 17:4916–4928

Al-Awadhi H, Al-Mailem D, Dashti N, Khanafer M, Radwan S (2012) Indigenous hydrocarbon-utilizing bacterioflora in oil-polluted habitats in Kuwait, two decades after the greatest man-made oil spill. Arch Microbiol 194:689–705

Ali N, Dashti N, Al-Mailem D, Eliyas M, Radwan S (2012) Indigenous soil bacteria with the combined potential for hydrocarbon consumption and heavy metal resistance. Environ Sci Pollut Res 19:812–820

Ali N, Dashti N, Salamah S, Sorkhoh N, Al-Awadhi H, Radwan S (2016a) Dynamics of bacterial populations during bench-scale bioremediation of oily seawater and desert soil bioaugmented with coastal microbial mats. Microb Biotechnol 9:157–171

Ali N, Dashti N, Salamah S, Al-Awadhi H, Sorkhoh N, Radwan S (2016b) Autochthonous bioaugmentation with environmental samples rich in hydrocarbonoclastic bacteria for bench-scale bioremediation of oily seawater and desert soil. Environ Sci Pollut Res 23:8686–8698

Al-Kharusi S, Abed RMM, Dobretsov S (2016a) Changes in respiration activities and bacterial communities in a bioaugmented oil-polluted soil in response to the addition of acyl homoserine lactones. Int Biodeterior Biodegrad 107:165–173

Al-Kharusi S, Abed RMM, Dobretsov S (2016b) EDTA addition enhances bacterial respiration activities and hydrocarbon degradation in bioaugmented and non-bioaugmented oil-contaminated desert soils. Chemosphere 147:279–286

Al-Kindi S, Abed RMM (2016a) Comparing oil degradation efficiency and bacterial communities in contaminated soils subjected to biostimulation using different organic wastes. Water Air Soil Pollut 227:36

Al-Kindi S, Abed RMM (2016b) Effect of biostimulation using sewage sludge, soybean meal, and wheat straw on oil degradation and bacterial community composition in a contaminated desert soil. Front Microbiol 7:240

Al-Mahruki A, Al Mueini R, Al-Mahrooqi Y, Al-Sabahi A, Roos GHP, Patzelt H (2006) Significantly enhanced landfarming performance through the use of saline water and weekly tilling.

In: Proceedings of the SPE international conference on health and safety, and environment in oil and gas exploration and production, Abu Dhabi, SPE-98568-PP

Al-Mailem DM, Kansour MK, Radwan SS (2015) Moderately thermophilic, hydrocarbonoclastic bacterial communities in Kuwaiti desert soil: enhanced activity via Ca^{2+} and dipicolinic acid amendment. Extremophiles 19:573–583

Al-Mueini R, Al-Dalali M, Al-Amri IS, Patzelt H (2007) Hydrocarbon degradation at high salinity by a novel extremely halophilic actinomycete. Environ Chem 4:5–7

Álvarez LM, Ruberto LA, Balbo AL, Mac Cormack WP (2017) Bioremediation of hydrocarbon-contaminated soils in cold regions: development of a pre-optimized biostimulation biopile-scale field assay in Antarctica. Sci Total Environ 590:194–203

Balba MT, Al-Daher R, Al-Awadhi N, Chino T, Tsuji H (1998) Bioremediation of oil-contaminated desert soil: the Kuwaiti experience. Environ Int 24:163–173

Barabás G, Sorkhoh NA, Fardoon F, Radwan SS (1995) n-Alkane-utilization by oligocarbophilic actinomycete strains from oil-polluted Kuwaiti desert soil. Actinomycetologica 9:13–18

Barabás G, Penyige A, Szabo I, Vargha G, Damjanovich S, Matko J, Szollosi J, Radwan SS, Matyus A, Hirano T (2000) Hydrocarbon uptake and utilization by *Streptomyces* strains. In: Wise DL, Trantolo DJ (eds) Remediation of hazardous wastes contaminated soils, 2nd edn. Marcel Dekker, New York, pp 291–309

Barabás G, Vargha G, Szabó IM, Penyige A, Damjanovich S, Szöllösi J, Matkó J, Hirano T, Mátyus A, Szabó I (2001) n-Alkane uptake and utilisation by *Streptomyces* strains. Anton Leeuw 79:269–276

Baraniecki CA, Aislabie J, Foght JM (2002) Characterization of *Sphingomonas* sp. Ant 17, an aromatic hydrocarbon-degrading bacterium isolated from Antarctic soil. Microb Ecol 43:44–54

Bej AK, Saul D, Aislabie J (2000) Cold-tolerant alkane-degrading *Rhodococcus* species from Antarctica. Polar Biol 23:100–105

Bell TH, Yergeau E, Martineau C, Juck D, Whyte LG, Greer CW (2011) Identification of nitrogen-incorporating bacteria in petroleum-contaminated arctic soils by using [15N] DNA-based stable isotope probing and pyrosequencing. Appl Environ Microbiol 77:4163–4171

Cho BN, Chino H, Tsuji H, Kunito T, Nagaoka K, Otsuka S, Yamashita K, Matsumoto S, Oyaizu H (1997a) Laboratory-scale bioremediation of oil-contaminated soil of Kuwait with soil amendment materials. Chemosphere 35:1599–1611

Cho BN, Chino H, Tsuji H, Kunito T, Makishima H, Uchida H, Matsumoto S, Oyaizu H (1997b) Analysis of oil components and hydrocarbon-utilizing microorganisms during laboratory-scale bioremediation of oil-contaminated soil of Kuwait. Chemosphere 35:1613–1621

Dashti N, Al-Awadhi H, Khanafer M, Abdelghany S, Radwan S (2008) Potential of hexadecane-utilizing soil-microorganisms for growth on hexadecanol, hexadecanal and hexadecanoic acid as sole sources of carbon and energy. Chemosphere 70:475–479

Dashti N, Khanafer M, El-Nemr I, Sorkhoh N, Ali N, Radwan S (2009) The potential of oil-utilizing bacterial consortia associated with legume root nodules for cleaning oily soils. Chemosphere 74:1354–1359

Dashti N, Ali N, Eliyas M, Khanafer M, Sorkhoh NA, Radwan SS (2015) Most hydrocarbonoclastic bacteria in the total environment are diazotrophic, which highlights their value in the bioremediation of hydrocarbon contaminants. Microbes Environ 30:70–75

Delille D, Coulon F, Pelletier E (2004a) Biostimulation of natural microbial assemblages in oil-amended vegetated and desert sub-Antarctic soils. Microb Ecol 47:407–415

Delille D, Coulon F, Pelletier E (2004b) Effects of temperature warming during a bioremediation study of natural and nutrient-amended hydrocarbon-contaminated sub-Antarctic soils. Cold Reg Sci Technol 40:61–70

Dias RL, Ruberto L, Hernández E, Vázquez SC, Balbo AL, Del Panno MT, Mac Cormack WP (2012) Bioremediation of an aged diesel oil-contaminated Antarctic soil: evaluation of the "on site" biostimulation strategy using different nutrient sources. Int Biodeterior Biodegrad 75:96–103

Eckford R, Cook FD, Saul D, Aislabie J, Foght J (2002) Free-living heterotrophic nitrogen-fixing bacteria isolated from fuel-contaminated Antarctic soils. Appl Environ Microbiol 68:5181–5185

El-Nawawy AS, Al-Daher R, Yateem A (1999) Plant–soil interaction during bioremediation of oil contaminated soil. In: Kostecki P, Behbehani M (eds) Assessment and remediation of oil contaminated soils. New Age International, Kuwait, pp 218–224
Embar K, Forgacs C, Sivan A (2006) The role of indigenous bacterial and fungal soil populations in the biodegradation of crude oil in a desert soil. Biodegradation 17:369–377
Eriksson M, Dalhammar G, Mohn WW (2002) Bacterial growth and biofilm production on pyrene. FEMS Microbiol Ecol 40:21–27
Farrell RL, Rhodes PL, Aislabie J (2003) Toluene-degrading Antarctic *Pseudomonas* strains from fuel-contaminated soil. Biochem Biophys Res Commun 312:235–240
Gerhardt P, Marquis RE (1989) Spore thermoresistance mechanisms. In: Smith I, Slepecky RA, Setlow P (eds) Regulation of prokaryotic development. American Society for Microbiology, Washington, DC, pp 43–63
Godoy-Faúndez A, Antizar-Ladislao B, Reyes-Bozo L, Camaño A, Sáez-Navarrete C (2008) Bioremediation of contaminated mixtures of desert mining soil and sawdust with fuel oil by aerated in-vessel composting in the Atacama Region (Chile). J Hazard Mater 151:649–657
Hassan N, Rafiq M, Hayat M, Shah AA, Hasan F (2016) Psychrophilic and psychrotrophic fungi: a comprehensive review. Rev Environ Sci Biotechnol 15:147–172
Hughes KA, Bridge P, Clark MS (2007) Tolerance of Antarctic soil fungi to hydrocarbons. Sci Total Environ 372:539–548
Kaplan CW, Kitts CL (2004) Bacterial succession in a petroleum land treatment unit. Appl Environ Microbiol 70:1777–1786
Kerry E (1990) Microorganisms colonizing plants and soil subjected to different degrees of human activity, including petroleum contamination, in the Vestfold Hills and MacRobertson Land, Antarctica. Polar Biol 10:423–430
Leahy JG, Colwell RR (1990) Microbial degradation of hydrocarbons in the environment. Microbiol Rev 54:305–315
Mac Cormack WP, Fraile ER (1997) Characterization of a hydrocarbon degrading psychrotrophic Antarctic bacterium. Antarct Sci 9:150–155
Mahmoud HM, Suleman P, Sorkhoh NA, Salamah S, Radwan SS (2010) The potential of established turf cover for cleaning oily desert soil using rhizosphere technology. Int J Phytorem 13:156–167
Makut MD, Ishaya P (2010) Bacterial species associated with soils contaminated with used petroleum products in Keffi town, Nigeria. Afr J Microbiol Res 4:1698–1702
Margesin R, Schinner F (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl Microbiol Biotechnol 56:650–663
Master ER, Mohn WW (1998) Psychrotolerant bacteria isolated from Arctic soil that degrade polychlorinated biphenyls at low temperatures. Appl Environ Microbiol 64:4823–4829
McKinnon M, Vine P (1991) Tides of war. IMMEL, London
Mohamed ME, Al-Dousary M, Hamzah RY, Fuchs G (2006) Isolation and characterization of indigenous thermophilic bacteria active in natural attenuation of bio-hazardous petrochemical pollutants. Int Biodeterior Biodegrad 58:213–223
Noble IR, Gitay H (1996) Deserts in a changing climate: impacts. In: Watson RT, Zinyowera MC, Moss RH, Dokken DJ (eds) Climate change: impact, adaptation and migration of climate change: scientific–technical analysis. Cambridge University Press, New York, pp 159–165
Noy-Meir I (1973) Desert ecosystems: environment and producers. Annu Rev Ecol Syst 4:25–51
Obuekwe CO, Al-Jadi ZK, Al-Saleh ES (2009) Hydrocarbon degradation in relation to cell-surface hydrophobicity among bacterial hydrocarbon degraders from petroleum-contaminated Kuwait desert environment. Int Biodeterior Biodegrad 63:273–279
Panicker G, Aislabie J, Saul D, Bej AK (2002) Cold tolerance of *Pseudomonas* sp. 30-3 isolated from oil-contaminated soil, Antarctica. Polar Biol 25:5–11
Pruthi V, Cameotra SS (1997) Production and properties of a biosurfactant synthesized by *Arthrobacter protophormiae*, an Antarctic strain. World J Microbiol Biotechnol 13:137–139
Radwan SS (1990) Gulf oil spill. Nature 350:456

Radwan S (1998) Hydrocarbon uptake by *Streptomyces*. FEMS Microbiol Lett 169:87–94
Radwan S (2008) Microbiology of oil-contaminated desert soils and coastal areas in the Arabian Gulf region. In: Patrice D, Chandra SN (eds) Microbiology of extreme soils, 13th edn. Springer, Berlin/Heidelberg, pp 275–298
Radwan S (2009) Phytoremediation for oily desert soils. In: Singh A, Kuhad RC, Ward OP (eds) Advances in applied bioremediation. Springer, Berlin/Heidelberg, pp 279–298
Radwan SS, Al-Muteirie AS (2001) Vitamin requirements of hydrocarbon-utilizing soil bacteria. Microbiol Res 155:301–307
Radwan SS, Sorkhoh NA (1997) A feasibility study on seeding as a bioremediation practice for the oily Kuwaiti desert. J Appl Microbiol 83:353–358
Radwan SS, Sorkhoh NA, Al-Hasan RH (1995a) Self-cleaning and bioremediation potential of the Arabian Gulf. In: Cheremisinoff P (ed) Encyclopedia of environmental control technology, vol 9. Gulf, Houston, pp 901–924
Radwan S, Sorkhoh N, Israa EN (1995b) Oil biodegradation around roots. Nature 376:302–302
Radwan SS, Sorkhoh NA, Fardoun F, Al-Hasan RH (1995c) Soil management enhancing hydrocarbon biodegradation in the polluted Kuwaiti desert. Appl Microbiol Biotechnol 44:265–270
Radwan SS, Al-Awadhi H, El-Nemr IM (2000) Cropping as a phytoremediation practice for oily desert soil with reference to crop safety as food. Int J Phytorem 2:383–396
Radwan SS, Al-Mailem DM, Kansour MK (2017) Calcium (II) – and dipicolinic acid mediated-biostimulation of oil-bioremediation under multiple stresses by heat, oil and heavy metals. Sci Rep 7:9534
Ravelet C, Krivobok S, Sage L, Steiman R (2000) Biodegradation of pyrene by sediment fungi. Chemosphere 40:557–563
Reyes-Bozo L, Antizar-Lalislao B, Sáez-Navarrete C, Godoy-Faúndeza A (2010) Bioremediation of TOCs present in fuel-contaminated desert mining soil and sawdust in the Atacama region (Chile). In: Proceedings of the annual international conference on soils, sediments, water and energy, vol 13, article 4. http://scholarworks.umass.edu/soilsproceedings/vol13/iss1/4
Ruberto LA, Vazquez S, Lobalbo A, Mac Cormack WP (2005) Psychrotolerant hydrocarbon-degrading *Rhodococcus* strains isolated from polluted Antarctic soils. Antarct Sci 17:47–56
Saadoun I, Mohammad MJ, Hameed KM, Shawaqfah MA (2008) Microbial populations of crude oil spill polluted soils at the Jordan–Iraq desert (the Badia region). Braz J Microbiol 39:453–456
Sanscartier D, Zeeb B, Koch I, Reimer K (2009) Bioremediation of diesel-contaminated soil by heated and humidified biopile system in cold climates. Cold Reg Sci Technol 55:167–173
Saul DJ, Aislabie JM, Brown CE, Harris L, Foght JM (2005) Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. FEMS Microbiol Ecol 53:141–155
Setlow B, Atluri S, Kitchel R, Koziol-Dube K, Setlow P (2006) Role of dipicolinic acid in resistance and stability of spores of *Bacillus subtilis* with or without DNA-protective α/β-type small acid-soluble proteins. J Bacteriol 188:3740–3747
Sorkhoh NA, Ghannoum MA, Ibrahim AS, Stretton RJ, Radwan SS (1990) Crude oil and hydrocarbon-degrading strains of *Rhodococcus rhodochrous* isolated from soil and marine environments in Kuwait. Environ Pollut 65:1–17
Sorkhoh NA, Ibrahim AS, Ghannoum MA, Radwan SS (1993) High-temperature hydrocarbon degradation by *Bacillus stearothermophilus* from oil-polluted Kuwaiti desert. Appl Microbiol Biotechnol 39:123–126
Sorkhoh NA, Al-Hasan RH, Khanafer M, Radwan SS (1995) Establishment of oil-degrading bacteria associated with cyanobacteria in oil-polluted soil. J Appl Microbiol 78:194–199
Sorkhoh NA, Ali N, Dashti N, Al-Mailem DM, Al-Awadhi H, Eliyas M, Radwan SS (2010a) Soil bacteria with the combined potential for oil utilization, nitrogen fixation, and mercury resistance. Int Biodeterior Biodegrad 64:226–231
Sorkhoh NA, Ali N, Salamah S, Eliyas M, Khanafer M, Radwan SS (2010b) Enrichment of rhizospheres of crop plants raised in oily sand with hydrocarbon-utilizing bacteria capable of hydrocarbon consumption in nitrogen free media. Int Biodeterior Biodegrad 64:659–664

Subhash Y, Yoon DE, Lee SS (2017) *Skermanella mucosa* sp. nov., isolated from crude oil contaminated soil. Anton Leeuw 13:1–8

Sutton NB, Maphosa F, Morillo JA, Al-Soud WA, Langenhoff AA, Grotenhuis T, Rijnaarts HH, Smidt H (2013) Impact of long-term diesel contamination on soil microbial community structure. Appl Environ Microbiol 79:619–630

Thomassin-Lacroix EJ, Yu Z, Eriksson M, Reimer KJ, Mohn WW (2001) DNA-based and culture-based characterization of a hydrocarbon-degrading consortium enriched from Arctic soil. Can J Microbiol 47:1107–1115

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

Thompson KT, Crocker FH, Fredrickson HL (2005) Mineralization of the cyclic nitramine explosive hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine by *Gordonia* and *Williamsia* spp. Appl Environ Microbiol 71:8265–8272

Torsvik V, Øvreås L (2008) Microbial diversity, life strategies, and adaptation to life in extreme soils. In: Patrice D, Chandra SN (eds) Microbiology of extreme soils, 13th edn. Springer, Berlin/Heidelberg, pp 15–43

Whyte LG, Bourbonniere L, Greer CW (1997) Biodegradation of petroleum hydrocarbons by psychrotrophic *Pseudomonas* strains possessing both alkane (alk) and naphthalene (nah) catabolic pathways. Appl Environ Microbiol 63:3719–3723

Whyte LG, Slagman SJ, Pietrantonio F, Bourbonniere L, Koval SF, Lawrence JR, Inniss WE, Greer CW (1999) Physiological adaptations involved in alkane assimilation at a low temperature by *Rhodococcus* sp. strain Q15. Appl Environ Microbiol 65:2961–2968

Whyte LG, Smits THM, Labbè D, Witholt B, Greer CW, Van Beilen JB (2002a) Gene cloning and characterization of multiple alkane hydroxilase systems in *Rhodococcus* strains Q15 and NRRL B16531. Appl Environ Microbiol 68:5933–5942

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

Yang S, Wen X, Zhao L, Shi Y, Jin H (2014) Crude oil treatment leads to shift of bacterial communities in soils from the deep active layer and upper permafrost along the China–Russia crude oil pipeline route. PLoS One 9:e96552

Yateem A (2013) Rhizoremediation of oil-contaminated sites: a perspective on the Gulf War environmental catastrophe on the State of Kuwait. Environ Sci Pollut Res 20:100–107

Yateem A, Balba MT, El-Nawawy AS, Al-Awadhi N (2000) Plants-associated microflora and the remediation of oil-contaminated soil. Int J Phytorem 2:183–191

Yateem A, Balba MT, Al-Shayji Y, Al-Awadhi N (2002) Isolation and characterization of biosurfactant-producing bacteria from oil-contaminated soil. Soil Sediment Contam 11:41–55

Yergeau E, Sanschagrin S, Beaumier D, Greer CW (2012) Metagenomic analysis of the bioremediation of diesel-contaminated Canadian high arctic soils. PLoS One 7:e30058

Yu Z, Stewart GR, Mohn WW (2000) Apparent contradiction: psychrotolerant bacteria from hydrocarbon-contaminated arctic tundra soils that degrade diterpenoids synthesized by trees. Appl Environ Microbiol 66:5148–5154

Surface and Subsurface Coal Environments: From Environmental Formation and Chemistry to Microbial Communities

10

Christopher R. Marks and Amy V. Callaghan

Contents

C. R. Marks
U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Groundwater, Watershed, and Ecosystem Restoration Division, Ada, OK, USA

A. V. Callaghan (✉)
Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA
e-mail: acallaghan@ou.edu

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_9

Abstract

Coal and coal extracts fuel a large portion of global trade by contributing to industries in energy production, infrastructure development, and chemical/material processing. The mining and recovery of these resources have tremendous economic and environmental impacts in coal-rich nations. Coal-associated habitats (e.g., coalbeds, coal mines, and spoil heaps) are highly complex ecosystems that support a wide array of microbial life and biogeochemical processes influenced by ancient and near-term hydrogeological properties. Subsurface formation waters support syntrophic assemblages of Bacteria and Archaea that cooperatively mineralize coal-derived organic material to form coalbed methane. Spoil heaps of surface extraction wastes contribute large masses of toxic metals and sulfide-rich minerals to top soils and aquatic habitats, driving microbial redox cycles that promote acidification and metal leaching. Microbiology is, therefore, intricately linked to the coal industry and plays critical roles in aspects ranging from production to remediation.

1 Introduction

Coal is a solid, combustible, fossil fuel source produced from ancient and predominantly plant-based materials that have undergone "coalification," a diagenetic process of physical, chemical, and biological transformations. Coals differ in their physicochemical properties because of variability in the chemical composition of tissues within given plant species, as well as the evolution of the plant community membership with time. These various plant-derived molecules are the starting material in the coal formation process. During coalification, the deposited organic matter undergoes changes that result in increased carbon content, vitrinite reflectance, and aromaticity, with concomitant decreases in elemental oxygen and relative moisture content (Strąpoć et al. 2011). The extent of these transformations defines the ranks of coal ranging from lignite ("brown coal") to subbituminous coal, bituminous coal, and anthracite (sometimes included with bituminous coal) (World Energy Council 2016).

1.1 Coal Uses

The complex organic nature and high energy content of coal have been leveraged for the development of multiple uses across several industries. Coal is used for electricity generation (steam coal), heating (industrial and residential), production of steel (coking coal), and as sources of liquid (i.e., synfuels) and gaseous fuels (i.e., syngas). Coal and coal extracts can also be used as precursors in the production of a variety of chemicals and materials (e.g., creosote oil, naphthalene, phenol, benzene, soap, aspirins, solvents, dyes, plastics, and fibers) (U.S. Energy Information Administration 2016; World Coal Association 2017). Among these processes, the predominant use of coal is for the production of electricity, which accounted for 33% of total U.S.

electricity generation in 2015 (U.S. Energy Information Administration 2016). In addition to these uses, coal reserves can be exploited as sources of unconventional natural gas, specifically as coalbed methane, which is discussed in more detail below (Mastalerz 2014).

1.2 Global Scale of Coal Reserves, Coalbed Methane Reserves, and Reserve Production

From a resource standpoint, the estimates of worldwide, existing, coal reserves are mainly defined by whether they are classified as proved (or measured) versus those that are probable (or indicative), wherein those that are "proved" are considered to be both technologically and economically recoverable (World Coal Association 2017). Coalbed methane reserves are similarly defined. For the year 2015, estimates of proved anthracite and bituminous reserves, versus those classified as subbituminous and lignite coal reserves, were 403,199 and 488,332 million tonnes, respectively (BP Statistical Review of World Energy 2016), totaling approximately 892 billion tonnes (BP Statistical Review of World Energy 2016; World Coal Association 2017). The majority of these reserves are located in the United States (~237 billion tonnes), Russia (~157 billion tonnes), China (~115 billion tonnes), India (~61 billion tonnes), and Germany (~40 billion tonnes) (BP Statistical Review of World Energy 2016). Recently, the global coalbed methane (CBM) resource inventory was estimated to be between 113 and 184 Tm^3, of which 42 Tm^3 was classified as recoverable using existing technologies (Mastalerz 2014). Based on end-of-2015 estimates of total, global, natural gas reserves (~186.9 Tm^3) (BP Statistical Review of World Energy 2016), the global CBM reserves represent a significant proportion of that total natural gas inventory (60.4–98.4%). Russia (80 Tm^3), the United States (49.2 Tm^3), and China (31.2 Tm^3) harbor the greatest total CBM reserves, and together, they account for approximately 87% of the global CBM inventory (Mastalerz 2014). Based on 2010 estimates, the global leaders in coalbed methane production are the United States (53.4 billion m^3), Canada (9.1 billion m^3), Australia (5.4 billion m^3), and China (1.4 billion m^3) (Mastalerz 2014).

During the past few years, however, there has been a shift in the distribution of world energy resource production. Beginning in 2015, total world coal production decreased for the first time since 2000, with a decrease of 221 million tonnes produced between 2014 and 2015 (International Energy Agency 2016). Specifically, 7,929.7 and 7,708.7 million tonnes of coal were produced in 2014 and 2015, respectively (International Energy Agency 2016). In part, this was attributed to an increase in the use of renewables and natural gas for electricity generation compared to previous years (International Energy Agency 2016). The year 2015 also marked the first time when global production of lignite, steam, and coking coal experienced declines (International Energy Agency 2016). Currently, China leads the world in coal production (~1,827 million tonnes oil equivalent; 47.7% of the total world production of 3,830 million tonnes), followed by the United States (~455 million

tonnes; 11.9% of the total) and India (~284 million tonnes; 7.4% of the total) (BP Statistical Review of World Energy 2016).

2 Coal-Associated Environments

2.1 Coal Chemistry and Maturity

As mentioned above, coals are grouped into ranks based upon physicochemical characteristics including thermal maturity, relative moisture, carbon content, aromaticity, the depositional history of the initial plant matter, and the extent this material is transformed during coalification. Unsurprisingly, the chemical properties of coals from different formations can be highly variable within a given rank based upon differences in the initial composition of the plant communities and the depositional history of the organic material. During coalification, plant materials undergo diverse physical, chemical, and biological changes and ultimately become inclusions within sedimentary rock during diagenesis. There are three major classes of these inclusions, known as macerals, which are categorized by the chemical nature of the initial plant tissues from which they were derived: (1) vitrinite, which is derived from woody lignin-rich residues from stems, branches, etc.; (2) liptinite, which is more recalcitrant and typically consists of aliphatic residues from waxes, resins, spores, etc.; and (3) inertinite, which is derived from both vitrinite and liptinite materials but contains a higher aromatic character. As the plant matter is subjected to increasing temperature, pressure, and depositional time, the organic residues undergo transformations (e.g., organic ring constituents are oxidized and fused through condensation and dehydration reactions) that result in an increase in the carbon elemental composition and aromaticity (Strąpoć et al. 2011). For a more extensive review of biogeochemical conversions involved in maceral formation and maturation processes, please see the review by Hatcher and Clifford (1997). Vitrinite reflectance, based upon the vitrinite maceral, is the measure of a coal's thermal maturity and is used to grade the organic matter in ranks. Coals within increasing maturity ranks can be characterized based on the loss of water content, decreased elemental oxygen content, and increased aromaticity. Thus, the deposited material "matures" into a highly combustible, organic-rich, sedimentary rock of greater heating value with increasing depositional depth and time.

2.2 Coalbed/Produced Water Features

Concomitant with the genesis and transformation of coal material is the impact of the type of deposited organic matter, the depositional environment, the extent of coalification, and other subsurface influences on the chemistry of coal formation water. Coalbed formation waters are typically characterized as having high concentrations of Na^+ and HCO_3^- (Van Voast 2003). Chloride concentrations vary based on historical marine influence, and they range (within selected formations) from

~10 meq/L in the Raton Basin to as high as ~100 meq/L in the Black Warrior, San Juan, Piceance, and Uinta Basins (Van Voast 2003 and references therein). Powder River Basin formation waters are markedly lower in chloride content (<1 meq/L) (Rice et al. 2008; Strąpoć et al. 2008a; Wawrik et al. 2012). All of these formations are similar in that they contain subbituminous to bituminous ranked coals and are associated with methane production (Van Voast 2003), with methane production in the Powder River Basin being exclusively biogenic in origin (Strąpoć et al. 2007; Rice et al. 2008). Thus, there appears to be a geochemical trend in methane-producing coal formation waters being typified by high concentrations of Na^+, HCO_3^-, and Cl^-, with very limiting concentrations of nitrate or sulfate (<1 meq/L). Further examination of the Powder River Basin formation waters suggest that an influx of meteoric water led to a general dilution and strong reduction in observed chloride concentrations relative to other methane-bearing coal seams (Rice et al. 2008; Strąpoć et al. 2008a). In contrast to the above basins, waters from formations containing similarly ranked coals, but not associated with methane production, were typified by high concentrations of Na^+, SO_4^{2-}, and HCO_3^-, with higher proportions of divalent cations (e.g., Ca^{2+} and Mg^{2+}). These comparisons indicate that a collection of biogeochemical events occur within the formation water prior to and/or during coalbed methane production and include biotic and abiotic sulfate reduction, enrichment of bicarbonate concentration, and precipitation-mediated depletion of calcium and magnesium cations (Van Voast 2003 and references within).

Surveys of organic constituents within formation waters produced from CBM wells in the Black Warrior, Powder River, San Juan, Tongue River, and Williston Basins revealed a diversity of aliphatic and aromatic compounds including polycyclic aromatic hydrocarbons (PAHs); alkylated monoaromatic hydrocarbons and phenols; N-, S-, and O-containing heterocyclic compounds; aromatic amines; and long-chain aliphatic hydrocarbons and fatty acids (Orem et al. 2007, 2014; Formolo et al. 2008). Alkylated PAHs were the most commonly observed organic compound class in the formation waters and were predominantly two-ringed molecules, ranging in combined abundance from ~20 to 100 μg/L. Various N- and S-heterocyclic compounds (e.g., quinolines and benzothiazoles) have been previously documented in groundwaters associated with lignite coals (Tatu et al. 2000; Bunnell et al. 2006; Maharaj et al. 2014). The presence of high molecular weight alkanes (up to C_{36}) and alkenes observed in lower-rank coal formation waters has been putatively linked to resinlike polymers such as those in liptinite macerals (Orem and Finkelman 2003; Orem et al. 2014). The solubility of these hydrophobic aliphatic molecules within the formation waters could potentially be enhanced by the desorption of other hydrophobic aromatic constituents. A further study by Orem and colleagues on the temporal dynamics of the formation water organic composition in the Powder River Basin (WY) suggested that groundwater depleted in total organic carbon from the surrounding sandstone member may be infiltrating the coal-bearing formation member as a result of the co-production of water with coalbed methane (Orem et al. 2007). From these studies, it is clear that the organic composition of these coal-associated aquifers is highly influenced by the thermal maturity and maceral composition of the corresponding coal, as well as potential groundwater influxes from the surrounding environment.

2.3 Coal Spoil Heap Features

In addition to subsurface coal mining (i.e., underground mining), coal reserves can also be exploited via surface mining. Surface mining is a broad term that encompasses the extraction of coal (or minerals) via strip mining, open-pit mining, mountain-top removal mining, or high-wall mining. In all of these scenarios, the soil and rock overlying the requisite coal seams are removed, and the collective excavation wastes are deposited/placed as "spoil heaps" (i.e., spoil tips, pit banks, etc.). These heaps consist of a heterogeneous mixture of fine- to coarse-grained sandstones, clays, shales, and coal seam materials (Jha and Singh 1991). Throughout history, and as a result of advancements in mining technology, the heights and volumes of spoil heaps have increased since the twelfth century (Debehault 1968), and today, there are spoil heaps that can reach almost 200 m in height. Given the structural heterogeneity, spoil heaps display complex geomorphology, which is influenced by runoff and infiltration, soil compaction, acidification, and spontaneous self-combustion (Nyssen and Vermeersch 2010). Consequently, spoil heaps can also vary significantly in composition and chemistry. Compared to older reclaimed sites, younger spoil heaps are typically characterized by low carbon content and low biological activity (Schaefer et al. 1979; Insam and Domsch 1988; Frouz et al. 2001; Frouz and Novakova 2005; Elhottová et al. 2006; Baldrian et al. 2008; Urbanová et al. 2011), and the surfaces are usually oxic, with anoxic or suboxic conditions dominating below (Johnson 2003). Additionally, because coals can contain $\geq$10% sulfur as pyrite and/or as other metal sulfides, the oxidation of these reduced sulfide minerals as they are brought to the surface during mining processes leads to acid production, which contributes to acid mine drainage (Banks et al. 1997; Johnson 2003). Spoil heap drainage water, therefore, can range from being extremely acidic to alkaline, depending on whether the spoil material contains neutralizing components, such as carbonate minerals (e.g., calcite and dolomite) (Banks et al. 1997; Johnson 2003; Akcil and Koldas 2006). Simultaneously, pyrite and other sulfide minerals may exist in association with As, Bi, Cd, Co, Cu, Ga, In, Hg, Mo, Pb, Re, Sb, Se, Sn, Te, and Zn, and thus, the oxidation of pyrite and other sulfide minerals in these systems can also contribute to the leaching of heavy metals (for review see Banks et al. 1997). Although not covered in this review, the activity of acidophilic sulfide-oxidizing microorganisms plays an important role in acid mine drainage (AMD) via the chemical oxidation of iron sulfides (Baker and Banfield 2003; Méndez-García et al. 2015).

3 Microbiology of Coal Environments

3.1 Microbial Ecology of Coalbed Subsurface Environments

To date, research into the microbiology of coal environments has primarily focused on understanding the ecology and physiology of resident anaerobic communities and their role in biogenic coalbed (i.e., subsurface) methane production, with a majority

of the studies being conducted in North America. Isotopic analysis of $\delta^{13}C$ and δD of CO_2 and CH_4, as well as the proportions of C_1 vs. C_2 and C_3 gases within formations, are used to distinguish between thermogenic and biogenic origins of coalbed methane (CBM) (Schoell 1980; Rice 1993; Whiticar 1999). These data, in conjunction with microbiological investigations, can be leveraged to assess the putative methanogenic pathway(s) responsible for biological CBM production within a coalbed system (Whiticar et al. 1986; Botz et al. 1996; Conrad 2005). Based upon these analyses, biogenic methane has been identified within numerous coalfield systems (reviewed in Strapoć et al. 2011) as either the sole source (e.g., Powder River Basin) (Flores et al. 2008) or, more commonly, as a mixture with thermogenic methane (e.g., Hokkaido Island) (Shimizu et al. 2007).

As mentioned previously, waters in formations associated with methane production, both biogenic and thermogenic in origin, are deplete in sulfate and nitrate (<0.5 mM) as compared to those not shown to harbor significant stores of methane (Van Voast 2003). While previous pure culture studies on select members of the *Desulfovibrio* spp. (Ingvorsen and Jørgensen 1984) and *Thermodesulfovibrio* spp. (Sonne-Hansen et al. 1999) have identified kinetic half saturation constants for sulfate uptake (K_m 3 and 77 μM) in this range, the repeated identification of obligate methanogenic taxa (discussed below) and the *in situ* biological production of significant quantities of methane in these habitats (e.g., Powder River Basin) indicate that methanogenesis is the dominant terminal electron-accepting process employed in Na^+-HCO_3^--Cl^--type formation water habitats. Under these conditions, organic substrates leached from the coal-bearing strata into the formation water can be mineralized to CO_2 and CH_4 through syntrophically-mediated oxidation linked to hydrogenotrophic methanogenesis. Under the electron-accepting conditions of these habitats, the microorganisms occupying the primary trophic level and utilizing these coal-derived substrates will exhibit a fermentative metabolism yielding volatile fatty acids (e.g., acetate and propionate), molecular hydrogen and/or formate, and CO_2 as their major metabolic end products. This fermentation is constrained thermodynamically, only becoming favorable when the concentrations of molecular hydrogen and/or formate are kept below a critical threshold. Thus, these primary fermentative taxa must form syntrophic associations with hydrogenotrophic microorganisms to overcome their thermodynamic limitations. The physiological and ecological implications of syntrophy are reviewed by McInerney and colleagues (2008, 2009, 2011). Based upon these constraints, the complete mineralization of these coal-derived organics would require multiple functional groups of organisms participating in the syntrophically mediated fermentations and methanogenesis from the hydrogenotrophic, acetoclastic, and methoxydotrophic methanogenesis pathways. Models for this type of community function have been proposed for the methanogenic mineralization of long-chain alkanes (Zengler et al. 1999; Wawrik et al. 2016). As shown in Fig. 1, the biodegradation of these various organic compound classes to CO_2 and CH_4 involves the combined activities of microorganisms from numerous taxonomic groups in communities such as those observed within formation water surveys and cultivation studies.

3.2 Microbial Community Membership and Function in Coalbed Formation Water-Associated Habitats

Since the early 2000s, there have been several studies examining the membership and structure of microbial communities within coalbeds and coalbed-associated formation waters from basins across the world (Shimizu et al. 2007; Klein et al. 2008; Krüger et al. 2008; Li et al. 2008; Strąpoć et al. 2008b; Midgley et al. 2010; Penner et al. 2010; Guo et al. 2012b; Singh et al. 2012; Wawrik et al. 2012). Surveys of produced water communities from low-rank coal fields in the Eastern Ordos Basin (China) and in Alberta, Canada are predominantly colonized by taxa affiliated with the gamma- and beta-classes of Proteobacteria (Penner et al. 2010; Guo et al. 2012b). No archaeal 16S rRNA genes were observed in the Canadian field study, which the authors postulated could have resulted from the detection limit of the DNA extraction and amplification methods and/or sorption of cells to the coal matrix. However, Guo and colleagues identified archaeal assemblages in the Eastern Ordos Basin that were exclusively populated by the Methanosarcinaceae, a lineage of acetoclastic and methylotrophic methanogens (Penner et al. 2010; Guo et al. 2012a, b). In contrast, a study of the Illinois Basin (Indiana, USA) formation water community showed bacterial taxa affiliated with the Alphaproteobacteria, Firmicutes, Bacteroidetes, and Spirochaetes, and the archaeal 16S rRNA gene sequences were solely affiliated with the obligately hydrogenotrophic *Methanocorpusculum* spp. (Strąpoć et al. 2008b). Wawrik et al. (2012) found that the produced waters from the San Juan Basin (New Mexico, USA) are colonized by members of both the Methanomicrobiales and Methanosarcinales communities with the capacity for hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis (Wawrik et al. 2012). In contrast, a study investigating the San Juan Basin and the Western Canadian Sedimentary Basin (Alberta, Canada) found that methanogens were abundant in the CBM-produced waters but absent in deep CBM cuttings (An et al. 2013). With regard to bacterial communities in produced waters of the San Juan Basin, Wawrik et al. (2012) identified a wide array of taxonomic groupings within the Thermoanaerobacterales, Clostridiales, Actinomycetales, and Deltaproteobacteria (Desulfovibrionales, Desulfuromonadales, and Syntrophobacterales). The surveys of the San Juan Basin and the Western Canadian Sedimentary Basin produced waters by An et al. (2013) identified anaerobic taxa, such as methanogenic Archaea and Clostridiales/*Sporomusa*, as well as aerobic and facultative lineages (e.g., Pseudomonadales/*Pseudomonas*, Rhizobiales/*Rhizobium*, and Alteromonadales/*Marinobacter*); surveys of the CBM cuttings contained Clostridiales/*Acetobacterium*, Fusobacteriales/*Ilyobacter*, and *Desulfomicrobium* (An et al. 2013).

Based on chemical analyses of organic constituents within coalbed formation waters (Orem et al. 2007, 2014; Formolo et al. 2008), there is a diverse range of substrates that may be subject to microbial metabolism. Figure 1 depicts potential routes of biological mineralization of organic compound classes that may be important in CBM ecosystems (see Sect. 2.2). As shown, there are a number of enzymatic mechanisms for the oxygen-independent activation of hydrocarbons that have been previously described in obligately anaerobic taxa. The succinate synthases, members

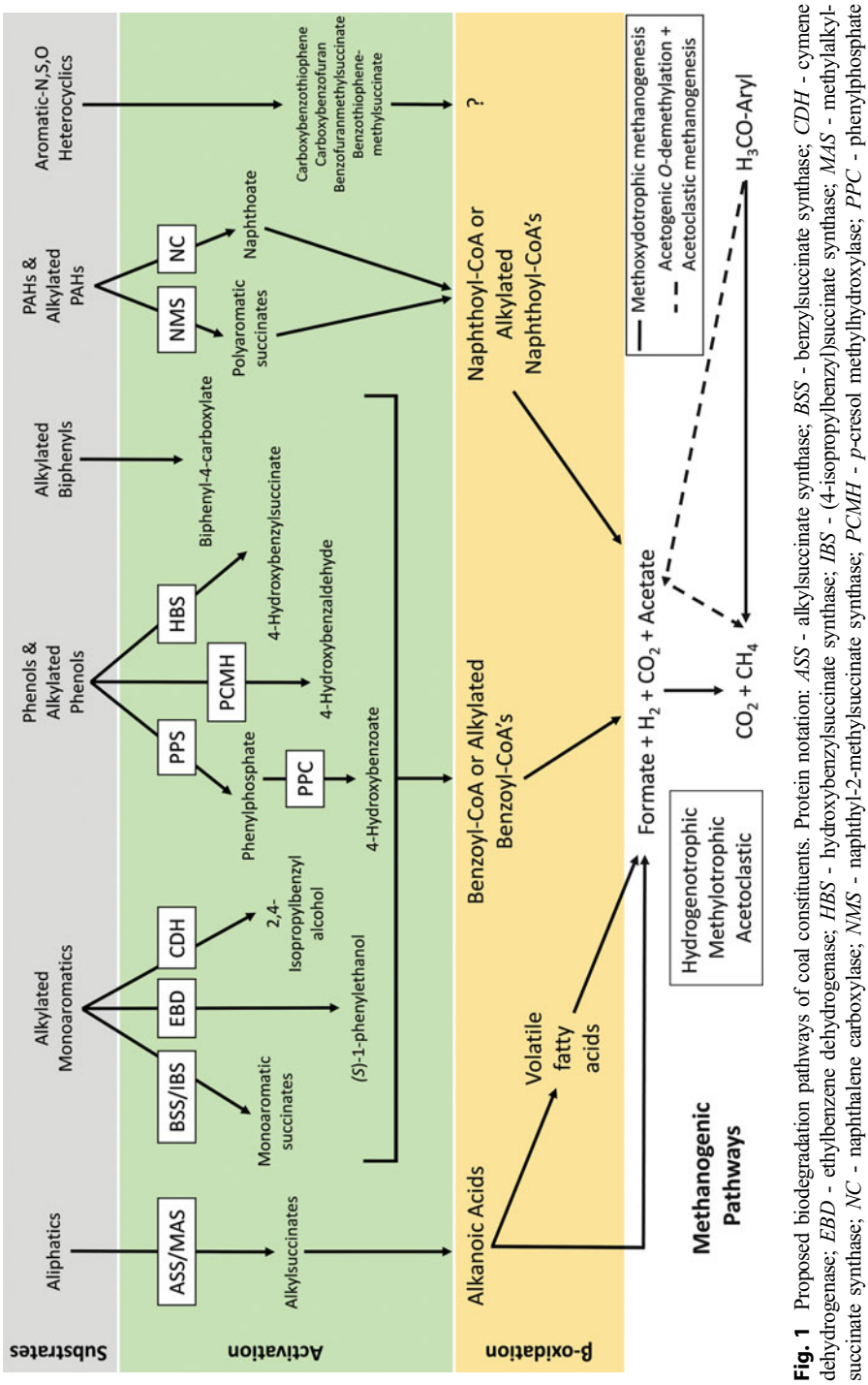

Fig. 1 Proposed biodegradation pathways of coal constituents. Protein notation: *ASS* - alkylsuccinate synthase; *BSS* - benzylsuccinate synthase; *CDH* - cymene dehydrogenase; *EBD* - ethylbenzene dehydrogenase; *HBS* - hydroxybenzylsuccinate synthase; *IBS* - (4-isopropylbenzyl)succinate synthase; *MAS* - methylalkyl-succinate synthase; *NC* - naphthalene carboxylase; *NMS* - naphthyl-2-methylsuccinate synthase; *PCMH* - *p*-cresol methylhydroxylase; *PPC* - phenylphosphate carboxylase; and *PPS* - phenylphosphate synthase

of the glycyl radical enzyme superfamily, are well characterized proteins that catalyze the addition of substrates to the double bond of fumarate ("fumarate addition"). The substrate range of these proteins include aliphatic hydrocarbons (alkylsuccinate synthase (ASS)/methylalkylsuccinate synthase (MAS)), monoaromatic and alkylated monoaromatic hydrocarbons (benzylsuccinate synthase (BSS) and 4-isopropylbenzylsuccinate synthase (IBS)), alkylated phenols (hydroxybenzylsuccinate synthase (HBS)), and polynuclear aromatic hydrocarbons ((2-methyl)naphthylsuccinate synthase (NMS)) (for review see Rabus et al. 2016). Anaerobic hydroxylation is another activation mechanism, and studies have shown that ethylbenzene, *p*-cymene, and *p*-cresol are hydroxylated via ethylbenzene dehydrogenase (EBD), cymene dehydrogenase (CMD), and *p*-cresol methylhydroxylase (PCMH), respectively (Keat and Hopper 1978; Hopper et al. 1991; Peters et al. 2007; Rabus et al. 2016). Alternatively, carboxylation has been observed as the first step in hydrocarbon activation of naphthalene by a naphthalene carboxylase and of benzene by anaerobic benzene carboxylase (for review and references therein, see Rabus et al. 2016). Phenol can be activated via two separate mechanisms: (i) the sequential activities of phenylphosphate synthase and phenylphosphate carboxylase, which have been documented in members of the Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria (Schmeling et al. 2004; Schleinitz et al. 2009; Schmeling and Fuchs 2009), and (ii) direct carboxylation to *p*-hydroxybenzoate by 4-hydroxybenzoate decarboxylase, which has been observed in members of the Clostridiales (Li et al. 2000).

To date, the information about these biochemical pathways has been used in genetic surveys and metabolite profiling to target the above processes in hydrocarbon-impacted environments. Specifically, the genes encoding the catalytic subunits of these enzymes (e.g., *assA*, *bssA*, *nmsA*, and *ebd*) and/or the requisite metabolites have been used as genetic and metabolic biomarkers (Callaghan et al. 2010; Agrawal and Gieg 2013; Callaghan 2013; von Netzer et al. 2013). With regard to subsurface coal environments, these approaches have been employed to further elucidate the potential metabolic pathways involved in the conversion of coal to methane. An investigation by Wawrik et al. (2012) detected *assA*, *tutD*, *bbsG*, and *ebd* gene signals within the Fruitland Coal Formation (San Juan Basin, NM) produced waters, suggesting the genetic potential for anaerobic transformation of alkanes and monoaromatics. A suite of genes involved in anaerobic benzoate degradation was also identified (*bclA*, *bcr*, *badK*, and *badH*). Detected metabolites in these produced waters included low molecular weight (C_2–C_9) fatty acids, saturated and unsaturated alkylsuccinic acids, naphthoate, tetrahydronaphthoate, benzoate, succinate, adipate, as well as toluic (*o*-, *m*-, and *p*-) and phthalic acids (*o*-, *m*-, and *p*-) and cresols (*m*- and *p*-). These metabolites are consistent with both aerobic and anaerobic biotransformation of coal constituents. Subsequently, another study (An et al. 2013) employed metagenomic analysis of CBM produced water, cuttings, and cores from the San Juan Basin and the Western Canadian Sedimentary Basin (Alberta, Canada), revealing the presence of *bssA* and other genes associated with the subsequent degradation of benzylsuccinate

(e.g., succinyl-CoA:(*R*)-benzylsuccinyl CoA-transferase, (*R*)-benzylsuccinyl-CoA dehydrogenase), along with phenylphosphate carboxylase (gamma subunit). These inventories support the genetic potential for anaerobic degradation of monoaromatic hydrocarbons and phenol. Interestingly, and consistent with taxonomic surveys of these samples (see Sect. 3.1), several genes identified as monooyxgenases and dioxygenases, which can be found in aerobic and facultative organisms, were also detected in these CBM samples, indicating the potential for aerobic degradation of hydrocarbon compounds (An et al. 2013).

In addition to genetic and metabolite surveys, there have also been a number of cultivation-based studies demonstrating the biological production of methane in incubations containing coal (Shumkov et al. 1999; Green et al. 2008; Harris et al. 2008; Krüger et al. 2008; Jones et al. 2010; Orem et al. 2010; Penner et al. 2010; Strąpoć et al. 2011; Ünal et al. 2012; Wawrik et al. 2012; Furmann et al. 2013). Recently, a study showed an axenic culture of the methanogen *Methermicoccus shengliensis* capable of directly producing methane from coal-derived organic material through a novel methanogenic pathway, methoxydotrophic methanogenesis (Mayumi et al. 2016). These studies reinforce the findings of DNA-based molecular surveys (discussed previously) that coal formation waters are colonized by viable and active methanogens. There have been repeated observations that aromatic compounds are preferentially degraded over aliphatic substrates in incubations with coal extracts (Orem et al. 2010; Furmann et al. 2013). These incubations also demonstrated biodegradation of N-, S-, and O-heterocyclic compounds during methanogenesis, suggesting that aqueous solubility and molecules with lower activation energies are key considerations in determining degradability of coal-extracted organic compounds. A few studies have been conducted to examine the impacts of various amendments in efforts to biostimulate or bioaugment methanogenic activity in incubations (Harris et al. 2008; Ulrich and Bower 2008; Jones et al. 2010). Separate incubation studies identified acetate as a critical intermediate in the conversion of coal-derived organic materials to methane, but exogenous addition of acetate did not stimulate enhanced methane production (Harris et al. 2008; Ulrich and Bower 2008). Autotrophic acetogenesis was observed in incubations when methanogenesis was inhibited, suggesting there may be competing fate processes for H_2/CO_2 available within formation waters (Harris et al. 2008). Biostimulation of methanogenesis was observed in incubations of subbituminous coal slurries amended with a defined growth medium (Jones et al. 2010), as well as those derived from Powder River Basin CBM water supplemented with a trace element solution (Ünal et al. 2012). Critical findings from these enrichment studies showed that the rates and extent of methanogenesis within coalbed ecosystems are dependent upon several factors, including thermal maturity, with lower-rank coals supporting greater rates (Strąpoć et al. 2011; Furmann et al. 2013; Lyles et al. 2017), increased surface area of coal exposed to formation waters (Green et al. 2008), and environmental conditions amenable to the growth of methanogens (e.g., pH, available trace nutrients, and absence of alternative electron acceptors such as nitrate/sulfate, etc.) (Shumkov et al. 1999; Green et al. 2008; Jones et al. 2010).

Ultimately, the various activation mechanisms and metabolic pathways associated with the wide array of potential organic substrates observed in formation waters (Fig. 1) lead to the formation of central catabolic intermediates (e.g., fatty acyl-CoAs, alkylated benzoyl-CoAs, and alkylated naphthoyl-CoAs) that are further oxidized via β-oxidation. Taken together, data from the various geological, chemical, genetic, and metabolic surveys and cultivation studies highlight the complexity of these subsurface ecosystems. Numerous ecological and physiological interactions impacted by the geological and geochemical characteristics of the formation material, as well as hydrological controls in formation recharge and re-inoculation, all culminate to result in the biological production of methane from coal-derived organic material.

3.3 Microbial Community Structure and Ecology of Spoil Heaps

To date, the majority of studies addressing the microbial ecology of coal environments have focused on coalbeds (see above) and the microbial composition and ecology of acid mine drainage waters derived from mining activities and spoil heap material (for reviews, see Baker and Banfield 2003 and Méndez-García et al. 2015). Few studies have investigated the microbial composition and function of the actual spoil heaps in depth (Kirby et al. 2010). These ecosystems are dynamic and are altered by microbial communities. As mentioned above (Sect. 2.3), younger spoil heaps are often nutrient-limited and exhibit low biological activity. The majority of microbial surveys have therefore focused on abandoned heaps, spontaneous succession of microbial communities in spoil heaps, and/or how microbial succession influences the soil development and soil properties of refuse piles (Frouz et al. 2001; Frouz and Novakova 2005; Baldrian et al. 2008; Urbanová et al. 2011; Maharana and Patel 2014). With respect to microbial community composition, these investigations and others have mainly used direct counts (e.g., DAPI), phospholipid fatty acid analysis (PLFA), and the most probable number technique, whereas studies of microbial function or activity have relied on bulk measurements of enzyme activity (e.g., amylase, invertase, protease, urease, phosphatase, and dehydrogenase), $^{14}CO_2$ uptake, and/or O_2 consumption (see below).

To the authors' knowledge, the earliest investigation of coal spoil heaps was a study that led to the isolation of the thermophilic archaeon, *Thermoplasma acidophilum*, from a burning coal refuse pile at the Friar Tuck mine in southwestern Indiana (Darland et al. 1970). The 40-year-old coal refuse pile at Friar Tuck, along with active mining sites at Blackfoot Mine no. 5 (Winslow, Indiana) and Peabody Coal Co. (Wilmington, Ill), were subsequently studied using the most probable number (MPN) technique to determine numbers of chemolithotrophic bacteria, heterotrophic bacteria, and fungi (Belly and Brock 1974). In general, the highest numbers of iron-oxidizing bacteria and the highest rates of $^{14}CO_2$ uptake were detected at or near the surface of spoil heaps, where O_2 is not limited. Uptake was also highest in samples that were 3–5 years old, versus older samples, suggesting that the older sites may have been depleted in nutrients and/or enriched with toxic

byproducts. Compared to heterotrophic bacteria counts, the fungi dominated the site surveys, resulting in the isolation of *Aureobasidium pullulans* and other fungi tentatively identified as *Penicillium* (Belly and Brock 1974).

More recently, phospholipid fatty acid analysis (PLFA), direct bacterial counts, and cultivable microbial counts were used to characterize microbial communities associated with spoil material from the Sokolov Brown Coal Basin (Elhottová et al. 2006). Although PLFA analysis only yielded nine fatty acids in two samples, total fatty acid profiles (TLFA) indicated a rich source of dead and stored biomass, which was proposed to serve as substrates for heterotrophic members. Fungi belonging to the genera *Penicillium*, *Verticillium*, *Cladosporium*, and *Aspergillus* were isolated, and bacterial isolates were predominantly ascribed to the genera *Nocardiopsis*, *Arthrobacter*, *Micrococcus*, *Kocuria*, *Rothia*, *Clavibacter*, *Bacillus*, *Paenibacillus*, *Brevibacillus*, *Microbacterium*, *Acinetobacter*, and *Pseudomonas*. Another study of the Sokolov brown coal mining district (Urbanová et al. 2011) examined a chronosequence of four post-mining sites (6, 12, 21, and 45 years old) that had undergone spontaneous succession. Soil properties changed with time indicating a decrease in pH and increase in the percentages of organic carbon and total nitrogen. PLFA-based estimates of microbial biomass indicated an increase between 6 and 21 years and then a decline for the 45-year old site. Among the interesting findings was the microbial community structure at the 6-year-old site. Based on 16S rRNA gene taxonomic microarrays, the surface soil (i.e., the top 5 cm) of the 6-year-old site was the most dissimilar from the other sites and was dominated by Gammaproteobacteria, Cyanobacteria, and some Alphaproteobacteria. Additionally, genera belonging to *Acidithiobacillus* and *Thiobacillus* were identified, which are among the most well-studied bacteria that play a role in AMD (for reviews, see Baker and Banfield 2003 and Méndez-García et al. 2015). Similarly, other studies have used PLFA (Baldrian et al. 2008; Maharana and Patel 2014) and randomly amplified polymorphic DNA (RAPD) analysis (Patel and Behera 2011) and/or have measured enzyme activities (Baldrian et al. 2008; Maharana and Patel 2013) to survey microbial communities in chronosequences of coal mine spoils. In general, these studies demonstrated that genetic diversity increased with the age of the spoil samples (Patel and Behera 2011) and that microbial enzyme activity increases with succession age (Baldrian et al. 2008; Maharana and Patel 2014).

Interestingly, despite advances in next-generation sequencing methodology, in-depth sequencing of 16S rRNA or 18S rRNA genes and/or metagenomic analyses have not been significantly exploited to survey spoil heaps. To date, there are very few examples in the literature. One study measured oxygen respiration rates and used 454-sequencing of 16S rRNA genes to examine microbial populations within undisturbed soil and overburden from a mining region that is part of the Wilds Conservation Center in Ohio (Meigs creek No. 9) (Poncelet et al. 2014). Spoil samples were collected from regions that were mined in 1973 and 1996. Microbial biomass estimates based on DNA yields and respiration rates increased with time. Phylogenetic analysis revealed that all three samples shared several dominant phylotypes often detected in soil systems (e.g., Burkholderiales). Both overburden samples contained Gammaproteobacteria related to *Stenotrophomonas*. The 1996

sample was characterized by a high abundance of *Limnobacter*-associated phylotypes, which are heterotrophic sulfur-oxidizing bacteria. In contrast, the 1973 overburden sample was dominated by *Ralstonia*- and *Rhodoferax*-affiliated phylotypes.

Although spoil heaps serve as the physical connection between surface mining activities and the development of AMD waters, the dearth of available studies cited above underscores the vast degree to which these systems have been understudied with respect to microbial communities and how they relate to reclamation efforts (see below).

4 Environmental Impact of Coal Mining

Regardless of whether coal is extracted via surface mining or underground mining, both processes exert a range of extensive environmental impacts associated with the excavation and/or extraction processes, as well as the downstream activities of coal preparation (e.g., sorting, crushing, cleaning, storage, homogenization, classification, dewatering, and desulfurization) and energy generation (Bian et al. 2010). Excavation processes ultimately reshape the landscape with respect to land use, loss or burial of native habitats, and land subsidence (Tiwary 2001). Collectively, these activities can contribute to the lowering of the water table (Tiwary 2001; Bian et al. 2010) and the alteration of watershed (Negley and Eshleman 2006; Ferrari et al. 2009; McCormick et al. 2009) and stream hydrology (Northington et al. 2011 and references therein). Runoff, drainage, and leaching from mining sites augment stream sediment loading (Tiwary 2001) and the contamination of surface water and groundwater systems. As discussed above, the oxidation of sulfur-bearing minerals in coal mines and spoil heaps generates acid with subsequent potential to leach co-occurring metals as "acid mine drainage" (AMD). Affected waters can experience significant decreases in pH and increases in conductivity, sulfate, iron, aluminum, and selenium, all of which affect aquatic wildlife (Tiwary 2001; Agouridis et al. 2012; Hopkins et al. 2013).

Coal mining also contributes to air pollution via the release of particulate matter and the emission of CO, CO_2, NOx, SOx, CH_4, and heavy metals (for reviews, see Ghose and Majee 2000b, c; Pandey et al. 2014). Dust emission is most often associated with surface mining due to drilling, blasting, coal and overburden loading/unloading, material transport, sizing and segregation, and disposal practices (Ghose and Majee 2000a; Ghose 2007). The release of CO, CO_2, NOx, and SOx results from coal burning, spontaneous combustion, and mine fires (Ghose and Majee 2000b; Bian et al. 2010), whereas the release of the potent greenhouse gas, methane, derives from coal seams, porous sandstone, fracture systems, joints, faults, and gas pockets (Lunarzewski 1998). In general, methane emissions are higher for underground mining compared to surface mining due to pressure and depth (Balat and Ayar 2004), and underground mining sites are characterized by instantaneous outbursts of both methane and carbon dioxide (Beamish and Crosdale 1998). These emissions not only pose a physical hazard, but they are a significant contributor to

greenhouse gas emissions (Limbri et al. 2013) and represent a significant waste with respect to energy recovery (Bian et al. 2010). Collectively, the industrial scale and worldwide distribution of coal extraction activities have yielded a legacy of these impacts on local, regional, and global ecosystems.

5 Research Needs

5.1 Future Directions

Coalbed methane remains a critical component of the energy economy. Some government and corporate entities continue to make investments in understanding the mechanisms of the biological conversion of coal to methane. As discussed in this chapter, coalbeds and coal-impacted environments are complex ecosystems with many critical connections between microbial community structure and function and reliable energy recovery and spoil reclamation efforts. With respect to the latter, there are still several gaps in our understanding that warrant further study.

5.2 Microbial Biotransformation of Methoxylated and Heterocyclic Aromatic Substrates in Subsurface Coal Environments

During the last two decades, great strides have been made in the elucidation of pathways and the characterization of proteins involved in the anaerobic metabolism of aliphatic and aromatic hydrocarbons. However, there is still a significant gap in our understanding regarding the anaerobic biotransformation of heterocyclic aromatic substrates, which are constituents of coal matrices. This is in part due to the vast structural diversity of these compounds. Previous studies have shown the anaerobic biodegradation of N-, S-, and O-containing aromatic compounds under a variety of electron-accepting conditions to be widely distributed across members of both the gram-positive and gram-negative lineages (Berry et al. 1987; Kuhn and Suflita 1989; Bollag and Kaiser 1991; Kaiser et al. 1996; Safinowski et al. 2006). Other studies have also shown that microbial consortia associated with aquifers can metabolize these compounds as substrates under both sulfate-reducing and methanogenic conditions (Kuhn and Suflita 1989; Annweiler et al. 2001; Safinowski et al. 2006). Experiments with the sulfate-reducing culture N47 revealed evidence that benzothiophene is activated via methylation and subsequent addition to fumarate to yield the carboxybenzothiophene derivative (Annweiler et al. 2001; Safinowski et al. 2006). To date, no defined genetic targets have been identified for the diagnostic detection of anaerobic thiophene metabolism. However, given that thiophenic compounds have been detected in CBM formation waters (see discussion in Sect. 2.2), these previous studies show that these compounds are biotransformed under anoxic conditions and may be expected to serve as substrates in coal seam waters.

In addition to expanding research about anaerobic transformation of heterocyclic compounds in coal environments, one of the most interesting findings in studies of the biological conversion of coal to methane is the direct production of methane from methoxylated substrates derived from coal by *Methermicoccus shengliensis* (Mayumi et al. 2016). This contrasts with the long-held model for methanogenesis from methoxylated aromatic substrates through the coupled processes of acetogenic *O*-demethylation and acetoclastic methanogenesis (Frazer 1994). The finding of methoxydotrophic methanogenesis and the availability of a model organism will enable continued investigations into the ecology of this process in coalbed formations, as well as the potential for biostimulation-/bioaugmentation-based methods for enhanced CBM recovery.

5.3 Methane Biofiltration for Coal Mine Ventilation Air

As mentioned above, fugitive, coal mine, methane emissions represent a physical hazard and are considered to be a significant contributor to greenhouse gas emissions (Limbri et al. 2013). Mine ventilation is used to dilute combustible gases in the mines and to keep methane concentrations below ignition limits (Limbri et al. 2013). During the past 25 years, there has been research to develop technologies that eliminate methane from the mine ventilation air (MVA) including combustion, gas concentration methods, and biofiltration. Combustion and gas concentration methods are considered to be expensive and have complex designs, large site requirements, and safety concerns (Limbri et al. 2013). The goal of methane biofiltration is to employ methanotrophic consortia to convert methane into biomass, CO_2, and water. This process has been used successfully to remove methane from environments such as landfills (Nikiema et al. 2007) and is being explored in the area of animal husbandry (Melse and van der Werf 2005). Although biofiltration may be more competitive cost-wise than combustion and gas concentration methods, it presents a host of additional challenges. The application of biofiltration to eliminate methane from MVA is influenced by temperature, moisture content, pH, nutrient content, packing material, low methane concentrations affecting microbial methane oxidation rates, and low methane solubility necessitating higher residence times (see Limbri et al. 2013 and references therein). The latter is considered a main driver for increasing operating costs. Altogether, these parameters have been, and continue to be, investigated for improving different types of biofilters. However, there is still a lack of understanding of whether biofilter performance with respect to MVA is governed more by mass transfer of methane or microbial activity (Limbri et al. 2013). With respect to methanotrophic consortia, the diversity of microbial species and their environmental requirements also preclude a "one-size-fits-all" solution to different coal environments. Despite these challenges, there have been recent studies to optimize and scale up methane-removing biofilters for pilot-scale use (Karthikeyan et al. 2017), which suggests that there may be a positive trajectory in this area of research.

5.4 Microbial Ecology of Reclamation

Although not extensively reviewed here, there have been several studies addressing the need to reconcile energy needs with the mitigation and/or remediation of coal-impacted environments via technical reclamation, spontaneous revegetation, and the development of "green mining" practices (Smyth and Dearden 1998; Bian et al. 2010; Sheoran et al. 2010). "Green mining" can encompass a range of techniques, such as water-preserved-mining, partial extraction and backfill mining, and underground coal gasification (Bian et al. 2010). The continued development and implementation of such efforts will ultimately be dependent on the world's future demand for coal. Technical reclamation and spontaneous revegetation of spoil heaps, however, reflect *current* remediation strategies to address the environmental legacy of coal mining. One of the greatest gaps in this area of research is the low resolution of microbial community analysis of microbial populations associated with reclaimed spoil heaps. Understanding the succession of microbial communities and biogeochemical processes during technical reclamation versus spontaneous revegetation could be significantly advanced by applying similar research strategies employed for subsurface coal-associated habitats including high-throughput sequencing, metabolomics, and directed cultivation studies. Information gained from these investigations may provide insight to strategies for the beneficial manipulation of the resident microbial communities within spoil heaps to mitigate detrimental impacts of AMD waters, which have a lasting effect on ecosystems, and to promote successful ecosystem restoration.

Acknowledgments The preparation of this chapter was funded in part by a National Science Foundation grant (MCB-1329890).

References

Agouridis CT, Angel PN, Taylor TJ, Barton CD, Warner RC, Yu X, Wood C (2012) Water quality characteristics of discharge from reforested loose-dumped mine spoil in Eastern Kentucky. J Environ Qual 41:454–468

Agrawal A, Gieg LM (2013) *In situ* detection of anaerobic alkane metabolites in subsurface environments. Front Microbiol 4:140

Akcil A, Koldas S (2006) Acid mine drainage (AMD): causes, treatment and case studies. J Clean Prod 14:1139–1145

An DS, Caffrey SM, Soh J, Agrawal A, Brown D, Budwill K et al (2013) Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. Environ Sci Technol 47:10708–10717

Annweiler E, Michaelis W, Meckenstock RU (2001) Anaerobic cometabolic conversion of benzothiophene by a sulfate-reducing enrichment culture and in a tar-oil-contaminated aquifer. Appl Environ Microbiol 67:5077–5083

Baker BJ, Banfield JF (2003) Microbial communities in acid mine drainage. FEMS Microbiol Ecol 44:139–152

Balat M, Ayar G (2004) Turkey's coal reserves, potential trends and pollution problems of Turkey. Energy Explor Exploit 22:71–81

Baldrian P, Trögl J, Frouz J, Šnajdr J, Valášková V, Merhautová V et al (2008) Enzyme activities and microbial biomass in topsoil layer during spontaneous succession in spoil heaps after brown coal mining. Soil Biol Biochem 40:2107–2115

Banks D, Younger PL, Arnesen RT, Iversen ER, Banks SB (1997) Mine-water chemistry: the good, the bad and the ugly. Environ Geol 32:157–174

Beamish BB, Crosdale PJ (1998) Instantaneous outbursts in underground coal mines: an overview and association with coal type. Int J Coal Geol 35:27–55

Belly RT, Brock TD (1974) Ecology of iron-oxidizing bacteria in pyritic materials associated with coal. J Bacteriol 117:726–732

Berry DF, Francis AJ, Bollag JM (1987) Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. Microbiol Rev 51:43–59

Bian Z, Inyang HI, Daniels JL, Otto F, Struthers S (2010) Environmental issues from coal mining and their solutions. Min Sci Technol 20:0215–0223

Bollag JM, Kaiser JP (1991) The transformation of heterocyclic aromatic compounds and their derivatives under anaerobic conditions. Crit Rev Environ Control 21:297–329

Botz R, Pokojski H-D, Schmitt M, Thomm M (1996) Carbon isotope fractionation during bacterial methanogenesis by CO_2 reduction. Org Geochem 25:255–262

BP Statistical Review of World Energy (2016) BP Statistical Review of World Energy 2016, 65th edn. London. https://www.bp.com/content/dam/bp/pdf/energy-economics/statistical-review-2016/bp-statistical-review-of-world-energy-2016-full-report.pdf

Bunnell JE, Tatu CA, Bushon RN, Stoeckel DM, Brady AMG, Beck M et al (2006) Possible linkages between lignite aquifers, pathogenic microbes, and renal pelvic cancer in northwestern Louisiana, USA. Environ Geochem Health 28:577–587

Callaghan AV (2013) Metabolomic investigations of anaerobic hydrocarbon-impacted environments. Curr Opin Biotechnol 24:506–515

Callaghan AV, Davidova IA, Savage-Ashlock K, Parisi VA, Gieg LM, Suflita JM et al (2010) Diversity of benzyl- and alkylsuccinate synthase genes in hydrocarbon-impacted environments and enrichment cultures. Environ Sci Technol 44:7287–7294

Conrad R (2005) Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. Org Geochem 36:739–752

Darland G, Brock TD, Samsonoff W, Conti SF (1970) A thermophilic, acidophilic mycoplasma isolated from a coal refuse pile. Science 170:1416–1418

Debehault C (1968) Les terrils de charbonnage du Borinage. Etude de géographie régionale. Rev Belge Géog 92:9–57

Elhottová D, Krištůfek V, Frouz J, Nováková A, Chroňáková A (2006) Screening for microbial markers in Miocene sediment exposed during open-cast brown coal mining. Antonie Van Leeuwenhoek 89:459–463

Ferrari JR, Lookingbill TR, McCormick B, Townsend PA, Eshleman KN (2009) Surface mining and reclamation effects on flood response of watersheds in the central Appalachian Plateau region. Water Resour Res 45:4

Flores RM, Rice CA, Stricker GD, Warden A, Ellis MS (2008) Methanogenic pathways of coal-bed gas in the Powder River Basin, United States: the geologic factor. Int J Coal Geol 76:52–75

Formolo M, Martini A, Petsch S (2008) Biodegradation of sedimentary organic matter associated with coalbed methane in the Powder River and San Juan Basins, U.S.A. Int J Coal Geol 76:86–97

Frazer AC (1994) *O*-Demethylation and Other Transformations of Aromatic Compounds by Acetogenic Bacteria. In: Drake HL (ed) Acetogenesis. Springer US, Boston, pp 445–483

Frouz J, Novakova A (2005) Development of soil microbial properties in topsoil layer during spontaneous succession in heaps after brown coal mining in relation to humus microstructure development. Geoderma 129:54–64

Frouz J, Keplin B, Pizl V, Tajovsky K, Stary J, Lukesova A et al (2001) Soil biota and upper soil layer development in two contrasting post-mining chronosequences. Ecol Eng 17:275–284

Furmann A, Schimmelmann A, Brassell SC, Mastalerz M, Picardal F (2013) Chemical compound classes supporting microbial methanogenesis in coal. Chem Geol 339:226–241

Ghose MK (2007) Generation and quantification of hazardous dusts from coal mining in the Indian context. Environ Monit Assess 130:35–45

Ghose MK, Majee SR (2000a) Assessment of dust generation due to opencast coal mining – an Indian case study. Environ Monit Assess 61:255–263

Ghose MK, Majee SR (2000b) Sources of air pollution due to coal mining and their impacts in Jharia coalfield. Environ Int 26:81–85

Ghose MK, Majee SR (2000c) Assessment of the impact on the air environment due to opencast coal mining – an Indian case study. Atmos Environ 34:2791–2796

Green MS, Flanegan KC, Gilcrease PC (2008) Characterization of a methanogenic consortium enriched from a coalbed methane well in the Powder River Basin, U.S.A. Int J Coal Geol 76:34–45

Guo H, Yu Z, Liu R, Zhang H, Zhong Q, Xiong Z (2012a) Methylotrophic methanogenesis governs the biogenic coal bed methane formation in Eastern Ordos Basin, China. Appl Microbiol Biotechnol 96:1587–1597

Guo H, Liu R, Yu Z, Zhang H, Yun J, Li Y et al (2012b) Pyrosequencing reveals the dominance of methylotrophic methanogenesis in a coal bed methane reservoir associated with Eastern Ordos Basin in China. Int J Coal Geol 93:56–61

Harris SH, Smith RL, Barker CE (2008) Microbial and chemical factors influencing methane production in laboratory incubations of low-rank subsurface coals. Int J Coal Geol 76:46–51

Hatcher PG, Clifford DJ (1997) The organic geochemistry of coal: from plant materials to coal. Org Geochem 27:251–274

Hopkins RL, Altier BM, Haselman D, Merry AD, White JJ (2013) Exploring the legacy effects of surface coal mining on stream chemistry. Hydrobiologia 713:87–95

Hopper DJ, Bossert ID, Rhodes-Roberts ME (1991) *p*-Cresol methylhydroxylase from a denitrifying bacterium involved in anaerobic degradation of *p*-cresol. J Bacteriol 173:1298–1301

Ingvorsen K, Jørgensen BB (1984) Kinetics of sulfate uptake by freshwater and marine species of *Desulfovibrio*. Arch Microbiol 139:61–66

Insam H, Domsch KH (1988) Relationship between soil organic-carbon and microbial biomass on chronosequences of reclamation sites. Microb Ecol 15:177–188

International Energy Agency (2016) Key Coal Trends: Excerpt From Coal Information. In: Coal Information. IEA Publishing, Paris, France, pp 1–12. www.iea.org/publications/freepublications/publication/KeyCoalTrends.pdf

Jha AK, Singh JS (1991) Spoil characteristics and vegetation development of an age series of mine spoils in a dry tropical environment. Vegetatio 97:63–76

Johnson DB (2003) Chemical and microbiological characteristics of mineral spoils and drainage waters at abandoned coal and metal mines. Water Air Soil Pollut 3:47–66

Jones EJP, Voytek MA, Corum MD, Orem WH (2010) Stimulation of methane generation from nonproductive coal by addition of nutrients or a microbial consortium. Appl Environ Microbiol 76:7013–7022

Kaiser JP, Feng YC, Bollag JM (1996) Microbial metabolism of pyridine, quinoline, acridine, and their derivatives under aerobic and anaerobic conditions. Microbiol Rev 60:483–498

Karthikeyan OP, Saravanan N, Cirés S, Alvarez-Roa C, Razaghi A, Chidambarampadmavathy K, Velu C, Subashchandrabose G, Heimann K (2017) Culture scale-up and immobilisation of a mixed methanotrophic consortium for methane remediation in pilot-scale bio-filters. Environ Technol 38:474–482

Keat MJ, Hopper DJ (1978) *p*-Cresol and 3,5-xylenol methylhydroxylases in *Pseudomonas putida* NCIB 9869. Biochem J 175:649–658

Kirby BM, Vengadajellum CJ, Burton SG, Cowan DA (2010) Coal, Coal Mines, and Spoil Heaps. In: Timmis KN (ed) Handbook of Hydrocarbon and Lipid Microbiology. Springer, Berlin/Heidelberg, pp 2277–2292

Klein DA, Flores RM, Venot C, Gabbert K, Schmidt R, Stricker GD et al (2008) Molecular sequences derived from Paleocene Fort Union Formation coals vs. associated produced waters: implications for CBM regeneration. Int J Coal Geol 76:3–13

Krüger M, Beckmann S, Engelen B, Thielemann T, Cramer B, Schippers A, Cypionka H (2008) Microbial methane formation from hard coal and timber in an abandoned coal mine. Geomicrobiol J 25:315–321

Kuhn EP, Suflita JM (1989) Microbial degradation of nitrogen, oxygen and sulfur heterocyclic compounds under anaerobic conditions – studies with aquifer samples. Environ Toxicol Chem 8:1149–1158

Li T, Juteau P, Beaudet R, Lepine F, Villemur R, Bisaillon JG (2000) Purification and characterization of a 4-hydroxybenzoate decarboxylase from an anaerobic coculture. Can J Microbiol 46:856–859

Li D, Hendry P, Faiz M (2008) A survey of the microbial populations in some Australian coalbed methane reservoirs. Int J Coal Geol 76:14–24

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 224:1566

Lunarzewski LW (1998) Gas emission prediction and recovery in underground coal mines. Int J Coal Geol 35:117–145

Lyles C, Parisi V, Beasley W, Van Nostrand J, Zhou J, Suflita J (2017) Elucidation of the methanogenic potential from coalbed microbial communities amended with volatile fatty acids. FEMS Microbiol Ecol 93:fix040

Maharaj SVM, Orem WH, Tatu CA, Lerch HE, Szilagyi DN (2014) Organic compounds in water extracts of coal: links to Balkan endemic nephropathy. Environ Geochem Health 36:1–17

Maharana JK, Patel AK (2013) Characterization of physico-chemical properties and their impact on enzyme activities in a chronosequence coal mine overburden spoil as biomarker of reclamation process. J Bacteriol Parasitol 4:1000174

Maharana JK, Patel AK (2014) Microbial community PLFA responses to ecosystem restoration in a chronosequence coal mine overburden spoil and implications of soil quality. Int J Curr Microbiol App Sci 3:45–71

Mastalerz M (2014) Coal Bed Methane: Reserves, Production, and Future Outlook. In: Letcher TM (ed) Future Energy: Improved, Sustainable, and Clean Options For Our Planet. Elsevier, London, pp 145–158

Mayumi D, Mochimaru H, Tamaki H, Yamamoto K, Yoshioka H, Suzuki Y et al (2016) Methane production from coal by a single methanogen. Science 354:222–225

McCormick BC, Eshleman KN, Griffith JL, Townsend PA (2009) Detection of flooding responses at the river basin scale enhanced by land use change. Water Resour Res 45:8

McInerney MJ, Struchtemeyer CG, Sieber J, Mouttaki H, Stams AJM, Schink B et al (2008) Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. Ann N Y Acad Sci 1125:58–72

McInerney MJ, Sieber JR, Gunsalus RP (2009) Syntrophy in anaerobic global carbon cycles. Curr Opin Biotechnol 20:623–632

McInerney M, Sieber J, Gunsalus R (2011) Microbial syntrophy: ecosystem-level biochemical cooperation. Microbe Mag 6:479–485

Melse RW, van der Werf AW (2005) Biofiltration for mitigation of methane emission from animal husbandry. Environ Sci Technol 39:5460–5468

Méndez-García C, Peláez AI, Mesa V, Sánchez J, Golyshina OV, Ferrer M (2015) Microbial diversity and metabolic networks in acid mine drainage habitats. Front Microbiol 6:475

Midgley DJ, Hendry P, Pinetown KL, Fuentes D, Gong S, Mitchell DL, Faiz M (2010) Characterisation of a microbial community associated with a deep, coal seam methane reservoir in the Gippsland Basin, Australia. Int J Coal Geol 82:232–239

Negley TL, Eshleman KN (2006) Comparison of stormflow responses of surface-mined and forested watersheds in the Appalachian Mountains, USA. Hydrol Proc 20:3467–3483

von Netzer F, Pilloni G, Kleindienst S, Krüger M, Knittel K, Grundger F, Lueders T (2013) Enhanced gene detection assays for fumarate-adding enzymes allow uncovering of anaerobic hydrocarbon degraders in terrestrial and marine systems. Appl Environ Microbiol 79:543–552

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

Northington RM, Benfield EF, Schoenholtz SH, Timpano AJ, Webster JR, Zipper C (2011) An assessment of structural attributes and ecosystem function in restored Virginia coalfield streams. Hydrobiologia 671:51–63

Nyssen J, Vermeersch D (2010) Slope aspect affects geomorphic dynamics of coal mining spoil heaps in Belgium. Geomorphology 123:109–121

Orem WH, Finkelman RB (2003) 7.08 - Coal Formation and Geochemistry. In: Holland HD, Turekian KK (eds) Treatise on Geochemistry. Elsevier, Oxford, pp 191–222

Orem WH, Tatu CA, Lerch HE, Rice CA, Bartos TT, Bates AL et al (2007) Organic compounds in produced waters from coalbed natural gas wells in the Powder River Basin, Wyoming, USA. Appl Geochem 22:2240–2256

Orem WH, Voytek MA, Jones EJ, Lerch HE, Bates AL, Corum MD et al (2010) Organic intermediates in the anaerobic biodegradation of coal to methane under laboratory conditions. Org Geochem 41:997–1000

Orem W, Tatu C, Varonka M, Lerch H, Bates A, Engle M et al (2014) Organic substances in produced and formation water from unconventional natural gas extraction in coal and shale. Int J Coal Geol 126:20–31

Pandey B, Agrawal M, Singh S (2014) Assessment of air pollution around coal mining area: emphasizing on spatial distributions, seasonal variations and heavy metals, using cluster and principal component analysis. Atmos Pollut Res 5:79–86

Patel AK, Behera N (2011) Genetic diversity of coal mine spoil by metagenomes using random amplified polymorphic DNA (RAPD) marker. Indian J Biotechnol 10:90–96

Penner TJ, Foght JM, Budwill K (2010) Microbial diversity of western Canadian subsurface coal beds and methanogenic coal enrichment cultures. Int J Coal Geol 82:81–93

Peters F, Heintz D, Johannes J, van Dorsselaer A, Boll M (2007) Genes, enzymes, and regulation of *p*-cresol metabolism in *Geobacter metallireducens*. J Bacteriol Parasitol 189:4729–4738

Poncelet DM, Cavender N, Cutright TJ, Senko JM (2014) An assessment of microbial communities associated with surface mining-disturbed overburden. Environ Monit Assess 186:1917–1929

Rabus R, Boll M, Heider J, Meckenstock RU, Buckel W, Einsle O et al (2016) Anaerobic microbial degradation of hydrocarbons: from enzymatic reactions to the environment. J Mol Microbiol Biotechnol 26:5–28

Rice DD (1993) Composition and Origins of Coalbed Gas. In: Law BE, Rice DD (eds) Hydrocarbons From Coal. American Association of Petroleum Geologists, Tulsa, pp 159–184

Rice CA, Flores RM, Stricker GD, Ellis MS (2008) Chemical and stable isotopic evidence for water/rock interaction and biogenic origin of coalbed methane, Fort Union Formation, Powder River Basin, Wyoming and Montana U.S.A. Int J Coal Geol 76:76–85

Safinowski M, Griebler C, Meckenstock RU (2006) Anaerobic cometabolic transformation of polycyclic and heterocyclic aromatic hydrocarbons: evidence from laboratory and field studies. Environ Sci Technol 40:4165–4173

Schaefer WM, Nielson GA, Dollhopf DT, Temple KL (1979) Soil genesis, hydrological properties, root characteristics, and microbial activity of 1-to 50-year-old stripmine spoils. Interagency Energy/Environment R&D Report. Environment R & D Program Report, USDA, EPA-600/7-79-100, 212pp

Schleinitz KM, Schmeling S, Jehmlich N, von Bergen M, Harms H, Kleinsteuber S et al (2009) Phenol degradation in the strictly anaerobic iron-reducing bacterium *Geobacter metallireducens* GS-15. Appl Environ Microbiol 75:3912–3919

Schmeling S, Fuchs G (2009) Anaerobic metabolism of phenol in Proteobacteria and further studies of phenylphosphate carboxylase. Arch Microbiol 191:869–878

Schmeling S, Narmandakh A, Schmitt O, Gad'on N, Schuhle K, Fuchs G (2004) Phenylphosphate synthase: a new phosphotransferase catalyzing the first step in anaerobic phenol metabolism in *Thauera aromatica*. J Bacteriol 186:8044–8057

Schoell M (1980) The hydrogen and carbon isotopic composition of methane from natural gases of various origins. Geochim Cosmochim Acta 44:649–661

Sheoran V, Sheoran AS, Poonia P (2010) Soil reclamation of abandoned mine land by revegetation: a review. Intl J Soil Sediment Water 3:13

Shimizu S, Akiyama M, Naganuma T, Fujioka M, Nako M, Ishijima Y (2007) Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. Geobiology 5:423–433

Shumkov S, Terekhova S, Laurinavichius K (1999) Effect of enclosing rocks and aeration on methanogenesis from coals. Appl Microbiol Biotechnol 52:99–103

Singh DN, Kumar A, Sarbhai MP, Tripathi AK (2012) Cultivation-independent analysis of archaeal and bacterial communities of the formation water in an Indian coal bed to enhance biotransformation of coal into methane. Appl Microbiol Biotechnol 93:1337–1350

Smyth CR, Dearden P (1998) Performance standards and monitoring requirements of surface coal mine reclamation success in mountainous jurisdictions of western North America: a review. J Environ Manag 53:209–229

Sonne-Hansen J, Westermann P, Ahring BK (1999) Kinetics of sulfate and hydrogen uptake by the thermophilic sulfate-reducing bacteria *Thermodesulfobacterium* sp. strain JSP and *Thermodesulfovibrio* sp. strain R1Ha3. Appl Environ Microbiol 65:1304–1307

Strąpoć D, Mastalerz M, Eble C, Schimmelmann A (2007) Characterization of the origin of coalbed gases in southeastern Illinois Basin by compound-specific carbon and hydrogen stable isotope ratios. Org Geochem 38:267–287

Strąpoć D, Mastalerz M, Schimmelmann A, Drobniak A, Hedges S (2008a) Variability of geochemical properties in a microbially dominated coalbed gas system from the eastern margin of the Illinois Basin, USA. Int J Coal Geol 76:98–110

Strąpoć D, Picardal FW, Turich C, Schaperdoth I, Macalady JL, Lipp JS et al (2008b) Methane-producing microbial community in a coal bed of the Illinois Basin. Appl Environ Microbiol 74:2424–2432

Strąpoć D, Mastalerz M, Dawson K, Macalady J, Callaghan AV, Wawrik B et al (2011) Biogeochemistry of microbial coal-bed methane. Annu Rev Earth Planet Sci 39:617–656

Tatu C, Orem W, Feder G, Finkelman R, Szilagyi D, Dumitrascu V et al (2000) Additional support for the role of the Pliocene lignite derived organic compounds in the etiology of Balkan endemic nephropathy. J Med Biochem 4:95–102

Tiwary RK (2001) Environmental impact of coal mining on water regime and its management. Water Air Soil Pollut 132:185–199

U.S. Energy Information Administration (2016). Use of Coal. http://www.eia.gov/energyexplained/index.cfm?page=coal_use. Accessed 3 Feb 2017

Ulrich G, Bower S (2008) Active methanogenesis and acetate utilization in Powder River Basin coals, United States. Int J Coal Geol 76:25–33

Ünal B, Perry V, Sheth M, Gomez-Alvarez V, Chin K-J, Nüsslein K (2012) Trace elements affect methanogenic activity and diversity in enrichments from subsurface coal bed produced water. Front Microbiol 3:175

Urbanová M, Kopecký J, Valášková V, Ságová-Marečková M, Elhottová D, Kyselková M et al (2011) Development of bacterial community during spontaneous succession on spoil heaps after brown coal mining. FEMS Microbiol Ecol 78:59–69

Van Voast WA (2003) Geochemical signature of formation waters associated with coalbed methane. AAPG Bull 87:667–676

Wawrik B, Mendivelso M, Parisi VA, Suflita JM, Davidova IA, Marks CR et al (2012) Field and laboratory studies on the bioconversion of coal to methane in the San Juan Basin. FEMS Microbiol Ecol 81:26–42

Wawrik B, Marks CR, Davidova IA, McInerney MJ, Pruitt S, Duncan KE et al (2016) Methanogenic paraffin degradation proceeds via alkane addition to fumarate by '*Smithella*' spp. mediated by a syntrophic coupling with hydrogenotrophic methanogens. Environ Microbiol 18:2604–2619

Whiticar MJ (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. Chem Geol 161:291–314

Whiticar MJ, Faber E, Schoell M (1986) Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation – isotope evidence. Geochim Cosmochim Acta 50:693–709

World Coal Association (2017) Coal. https://www.worldcoal.org/. Accessed 3 Feb 2017

World Energy Council (2016) World Energy Resources Interactive. https://www.worldenergy.org/data/resources/resource/coal/. Accessed 3 Feb 2017

Zengler K, Richnow HH, Rossello-Mora R, Michaelis W, Widdel F (1999) Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 401:266–269

Microbial Communities in Oil Shales, Biodegraded and Heavy Oil Reservoirs, and Bitumen Deposits

11

Lisa M. Gieg

Contents

Abstract

Oil shale (kerogen), light crude oil, heavy crude oil, and bitumen represent a sequence of fossil energy materials that range from "pre-oil" to "ultra-heavy oil" that were formed and/or then transformed over geological time in the Earth's subsurface. Microorganisms can inhabit all of the deposits harboring these materials and have played a key role in transforming light crude oil to ultra-heavy bitumen. A majority of our understanding about how microbial life exists within and influences these hydrocarbon-associated subsurface environments comes from studying crude oil reservoirs, while comparatively little is known for oil shale or bitumen reservoirs. This chapter summarizes the knowledge to

L. M. Gieg (✉)
Department of Biological Sciences, University of Calgary, Calgary, AB, Canada
e-mail: lmgieg@ucalgary.ca

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_4

date on the microbial communities and their putative metabolic activities within these distinct petroliferous deposits.

1 Introduction

It is now recognized that the Earth's deep subsurface can support thriving microbial ecosystems (Colwell and D'Hondt 2013), and subsurface fossil energy-bearing environments are no exception (Larter and Head 2014). These environments include hydrocarbon reservoirs in the form of coal, shale, and various grades of crude oil. A previous review on this topic was penned by Foght (2010) who clearly defined the properties of oil shales, heavy oil reservoirs, and bitumen/tar deposits that are the focus of this chapter, along with the knowledge to date (e.g., up to 2010) available on the microbial communities associated with these fossil energy-bearing systems. Here, a brief introduction to the formation of these petroliferous systems is provided along with a description of oil shales, heavy oils, and bitumen. Past literature is summarized, and updates on any progress in our understanding of microbial communities and their functions within these environments are provided. As recent review articles have summarized the now extensive body of literature on microbial communities within biodegraded oil reservoirs (e.g., Head et al. 2014), this environment will be overviewed here in a more cursory fashion.

2 Formation of Fossil Energy Reserves and Definition of Terms

The organic theory of petroleum formation is based on the premise that crude oil originated from ancient biomass of marine origin that was buried and transformed to various substances over geological time due to the increasing heat and pressure that resulted with increasing depth of burial (Hunt 1996). Here, the process of diagenesis occurred at a relatively low temperature (<50 °C) which began to convert biomolecules into organic matter called kerogen, which is considered a "pre-oil" substance. As the subsurface pressure and temperature increased (up to ~150 °C) with increasing depth of burial, this kerogen was transformed into what we recognize as crude oil hydrocarbons through a process known as catagenesis. At even higher temperatures (>150–200 °C), all carbon forms were converted to graphite or thermogenic gas via metagenesis (Hunt 1996). These conversions have thus yielded various forms of petroliferous materials buried within the Earth's subsurface. The materials discussed in this chapter represent a "continuum of hydrocarbon mixtures" that were generated over geological time by thermal, physical, and/or microbiological processes (Hunt 1996, Foght 2010). These range from a "pre-oil" state (oil shale) (Fig. 1a) that with enough heating and pressure are transformed to light crude oils (Fig. 1b) that are then altered via biodegradation to various extents to become heavy oil (Fig. 1c) and subsequently bitumen/tar (Fig. 1d).

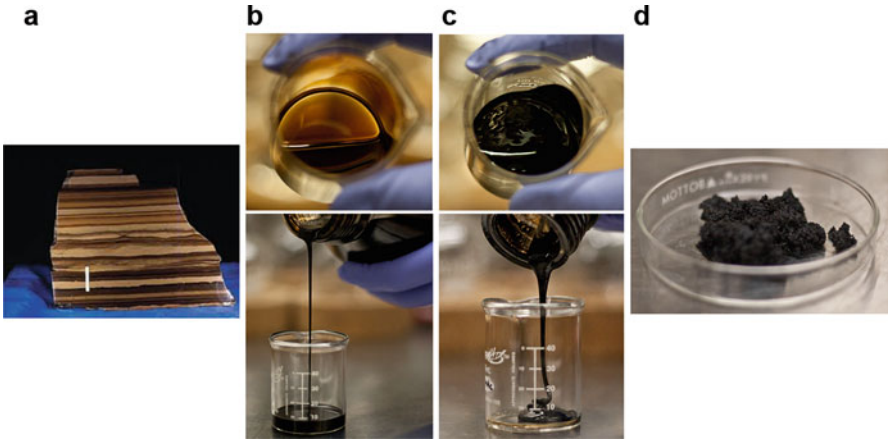

Fig. 1 Visualization of various petroliferous materials that can support microbial communities shown as a continuum from a "pre-oil" state to ultra-heavy biodegraded oils (left to right). (**a**) Oil shale kerogen that must undergo intense heating to be transformed to (**b**) light crude oil. Over geological time, low viscosity light oil is biodegraded to (**c**) heavy oil that becomes more viscous as light hydrocarbons are preferentially utilized by microorganisms. Extensive biodegradation can lead to the formation of (**d**) bitumen, an ultra-heavy oil (shown here as oil sands). (Photo credits: **a** – Sample of Green River oil shale (from Mahogany zone in the Piceance Creek Basin, Colorado), US Geological Survey; **b–d** – light oil, heavy oil, oil sands photos by Natalie Rachel (Gieg laboratory), University of Calgary)

3 Oil Shale

As indicated by Foght (2010), the term "oil shale" is a misnomer because this material does not actually contain oil but rather kerogen, which is considered as a "pre-oil" solid form of organic matter derived from ancient biomass. Dyni (2005) defines oil shale as a "fine-grained sedimentary rock containing solvent-insoluble organic matter that yields substantial amounts of oil and combustible gas upon destructive distillation"; in other words, oil shale comprises organic matter that must be further heated (to ~500 °C) in order to be transformed into a form of usable crude oil. Some oil shale deposits contain asphaltene-like solid hydrocarbons in proportionately small amounts that exist in pockets or veins – this oil shale component is often referred to as "bitumen," distinct from the bitumen resulting from extensive biodegradation of crude oil (described below). In contrast to kerogen, this "bitumen" component is organic solvent-soluble and can be fractionated to reveal some more oil-like saturate and aromatic components that may serve as substrates for microbial communities in subsurface environments (Meslé et al. 2015; Matlakowska and Sklodowska 2011). Oil shale deposits are found globally (Dyni 2005; Vandenbroucke and Largeau 2007), though the most well-known and largest oil shale deposit is found in the Green River Formation in the USA (underlying large parts of Wyoming, Utah, and Colorado) harboring an estimated 1.2 trillion barrels of

potentially recoverable oil (Johnson et al. 2010; US Geological Survey 2017). Despite the vast deposits of potential energy entrapped in oil shale, its recovery is currently not economically viable because of the extensive amount of heating (either aboveground or in situ) that is required to convert solid kerogen to a liquid hydrocarbon mixture (through a process known as retorting). It is very important to note here the difference between the terms "oil shale" and "shale oil" (Utah Geological Survey 2017). As already described, "oil shale" is kerogen that is difficult to recover and is considered a "pre-oil" requiring further processing by intense heating into a usable energy source, while "shale oil" (also known as "tight oil") is an already-formed liquid crude oil found in low-permeability rock formations (Mouser et al. 2016) that can be recovered by horizontal drilling and hydraulic fracturing. There is comparatively little information on microorganisms associated with oil shale deposits (kerogen), while over a dozen studies have now described microbial communities, and their potential functions, associated with low-permeability formations wherein hydraulic fracturing can be used to recover shale gas and shale oil (Mouser et al. 2016; Colosimo et al. 2016).

4 Heavy Oil

It has been stated that most of the world's crude oil reservoirs have been biodegraded to some extent (Roadifer 1987; Head et al. 2003), yielding various degrees of heavy oil. Crude oil hydrocarbons that are generated through catagenic processes are expelled from their deep, low-permeability source rocks and migrate upward through porous rock (e.g., sandstone or limestone) until they reach nonporous cap rocks that prevent further migration, yielding vast reservoirs of crude oil (and gas). Through this migration process that occurs over geological time, crude oil is subject to a variety of processes such as water washing, volatilization, and biodegradation, all of which alter the chemical (hydrocarbon) composition of crude oil by removing lighter hydrocarbons such that the crude oils become enriched in heavier hydrocarbons (Zhou et al. 2008). With these transformations, crude oils also become enriched in sulfur and metal content and become more acidic and viscous, leading to a lower quality crude oil that is difficult to recover and that requires additional refining procedures (Larter and Head 2014). Further biodegradation can occur as crude oil accumulates in a reservoir rock if conditions are favorable for microbial metabolism (see below). However, high temperatures and salinities have likely played major roles in limiting the extent of crude oil biodegradation (Head et al. 2014). While reservoirs lower than 80 °C typically harbor biodegraded oils (Head et al. 2003), in some cases they do not, which may have been due to a process known as "paleopasteurization" wherein crude oils were sterilized at high temperatures prior to being uplifted into lower temperature zones (Wilhelms et al. 2001). A similar concept with regard to salinity has recently been proposed (deemed "paleopickling") wherein high formation water salinity may have also prevented in situ crude oil biodegradation (Head et al. 2014).

The physical state of a crude oil (e.g., whether it is considered "light" or "heavy") is often characterized using a measure developed by the American Petroleum Institute (API) termed API gravity (°API; a measure of fluid density compared to that of water), wherein light oils have an °API > 32, medium oils are characterized by °API values ranging from 20–31, heavy oils range from 10–20 °API, and oils below 10 °API are considered ultra-heavy (such as bitumen, below; Hunt 1996). Some useful scales have been devised such as the Peters and Moldowan (PM) scale (1993) or the newer Manco scale (Larter et al. 2012) that relate the chemical composition to the quality of a crude oil. For example, an oil characterized by a PM = 1 or 2 is rich in low-molecular-weight hydrocarbons and is considered non-degraded, while oils with PM values >6 are considered heavily or severely biodegraded containing primarily recalcitrant multi-ringed polycyclic aromatic hydrocarbons (PAH), asphaltenes, and biomarkers such as hopanes and steranes (Head et al. 2003).

5 Bitumen

Bitumen is a term that is most commonly used to describe ultra-heavy oils that have been formed over geological time due to physiochemical weathering and extensive microbial degradation. While bitumen deposits can be found worldwide, the most economically viable deposits are found in the Orinoco belt of Venezuela and in the province of Alberta, Canada (Larter and Head 2014). Bitumen is a type of ultra-heavy crude oil that has an API gravity <10° and a viscosity of 10,000 centipoises or greater, meaning that it does not flow like a conventional light or heavy crude oil under ambient temperature and pressure conditions (Larter and Head 2014). Compared to heavy oil, bitumen is even more devoid of low molecular compounds and enriched in sulfur content, metals, and consists primarily of asphaltenes. Although no single asphaltene structure has been clearly elucidated, this crude oil fraction is known to contain highly heterogeneous condensed multi-cyclic aromatic compounds intertwined with alkyl side chains and enriched in heteroatoms (Selucky et al. 1977; Strausz et al. 1992). Natural asphalt and tar seeps are similarly comprised largely of asphaltenes.

6 Microbial Communities Associated with Oil Shale/Kerogen Deposits

Foght (2010) previously noted that there were few studies in the peer-reviewed literature describing microbial communities associated with oil shale (kerogen) deposits. Though this statement remains true, a few new reports have emerged describing microbial communities and activities associated with kerogen deposits, with studies having been primarily conducted with black shales in the eastern USA (e.g., New Albany or Antrim shale formations), or European shallow kerogen/black shale deposits as described below.

Early work with oil shale and its microbiology examined the prospects for using microbes that were either indigenous to or exogenously added to oil shales in order to help solubilize kerogen and/or remove sulfur in the interest of developing an economic method for extracting oil shale (Findley et al. 1974; Meyer and Yen 1976; Pfister et al. 1991). Both Findley et al. (1974) and Meyer and Yen (1976) incubated samples collected from the Green River oil shale basin with organisms such as sulfuric acid-producing *Thiobacillus* strains in order to increase porosity within oil shale material and/or remove sulfur components. The results of both studies demonstrated increased kerogen mobility through creating enhanced porosity, but the potential technology does not appear to have been pursued for these Green River oil shale materials. Rather than using exogenously added bacteria, Pfister et al. (1991) examined New Albany oil shale (from Indiana, USA) for the presence of indigenous microbial communities that may be used to remove sulfur. These authors were the first to demonstrate that oil shale materials harbored microbial communities that were present from 10^6 to 10^{10} cells/g shale (depending on the enumeration technique applied). They isolated several aerobic bacterial strains (though not identified) and showed that mixed cultures enriched from the oil shale in sulfur-free culture medium could significantly reduce the sulfur component of the oil shale (Pfister et al. 1991).

Petsch et al. (2001) subsequently conducted a series of studies using exposed New Albany shale materials (from an outcrop near Clay City, Kentucky; this black shale formation spans Indiana, Illinois, and Kentucky, USA) that showed different signs of kerogen weathering; microbial contribution to such weathering was unknown. As such, they sought to determine the contribution of indigenous microbial communities to utilize recalcitrant kerogen organic matter (OM) as a carbon source by establishing enrichments from the shale material that served as both the source of microbes and carbon. Using sensitive isotopic measurements (^{13}C- and ^{14}C-PLFA analysis) of the OM and microbial biomass, the authors unequivocally demonstrated that microorganisms associated with kerogen deposits utilized components of this refractory OM. This study offered previously unrecognized evidence that microbes indigenous to sedimentary shale rock can play a key role in global cycles of carbon and oxygen (Petsch et al. 2001). A subsequent study along a gradient of the New Albany shale material containing various degrees of OM (e.g., non-weathered to weathered, accessed via an outcrop) further revealed (based on PLFA analysis and clone libraries prepared from the 16S rRNA genes) that diverse aerobes and anaerobes are present and likely contribute to kerogen decomposition (Petsch et al. 2003).

Understanding how microorganisms are entrained in subsurface kerogen deposits has huge implications in energy production, particularly for natural gas production, e.g., stimulating microbial communities to produce methane. Here, mixed anaerobic microbial communities can feasibly utilize entrained, complex OM (including the "bitumen" fraction that may contain recognizable hydrocarbons) in a series of reactions that produce methanogenic substrates like H_2 and acetate, ultimately leading to hydrogenotrophic or acetotrophic methanogenesis (Schlegel et al. 2013; Meslé et al. 2012; Colosimo et al. 2016). A number of factors can influence in-rock microbial activity leading to methane including anaerobicity, quality of the entrained

OM, salinity, pH, nutrient availability (e.g., N, P, trace elements), water content, and porosity (McIntosh et al. 2002; Schlegel et al. 2013; Mouser et al. 2016; Colosimo et al. 2016). Methane from some shale formations (e.g., the Michigan Antrim shale formation) has been shown to possess a substantial biological signature as determined using isotopic measurements (Martini et al. 1996, 2008) which has led to the biotechnological prospect for stimulating methanogenesis from shale formations as a bioenergy recovery strategy. In contrast, when Schlegel et al. (2013) measured the labile OM content (e.g., alkanes, fatty acids, alcohols, etc.) in a large number of formation water and core samples from a part of the New Albany shale, they did not find strong evidence for biodegradation of shale components (possibly due to low biomass, increasing salinity, low nutrient availability, or pore space limitations in the area sampled). This demonstrates that there can be large differences in the abilities and rates of indigenous microbial communities to utilize OM associated with kerogen deposits, likely due to the associated geochemical environment.

To examine the potential for methanogenesis from kerogen, Meslé et al. (2012) used shallow immature shale material (ranging from 7.8 to 12 m below surface, mbs) from the eastern Paris basin; the kerogen was noted to be of Type II and to contain compounds having aromatic and naphthenic rings and aliphatic characteristics. Pulverized kerogen rock (prepared from samples collected every 10 cm) was incubated in strictly anoxic minimal salts medium containing different additives to determine whether methanogenic communities could be cultivated from the kerogen samples. Methane monitoring showed that materials collected from the 12 m depth produced methane within about 100 days (no methane was detected from materials from other depths), which correlated with an increased abundance of methanogens as determined using qPCR for the methanogen-specific *mcr*A gene (Meslé et al. 2012). Using clone libraries and 454 pyrosequencing of the 16S rRNA gene, methanogens were identified that most closely affiliated with members of the genus *Methanosarcina.* Bacteria within the methane-producing incubations were primarily members of the *Firmicutes*, with *Clostridium* phylotypes being most dominant. These organisms are known fermenters and likely were the key bacteria utilizing kerogen organic matter in concert with *Methanosarcina*, members of which can use diverse substrates for methanogenesis (e.g., they can be hydrogenotrophs, acetotrophs, or methylotrophs). Interestingly, sequencing efforts showed similar microbial taxa even in incubations that did not show methane production but contained primarily *Methanoculleus* (hydrogenotrophic) rather than the more versatile *Methanosarcina*. The authors postulated that this difference in the type of methanogen present affected methane production from kerogen decomposition but articulated that more studies are required. Nevertheless, these results indicated that kerogen type II organic matter can drive methanogenesis in immature shale rock. A follow-up study by Meslé et al. (2015) aimed to determine which fraction within the kerogen was serving as the carbon and energy source for extant methanogenic consortia. They compared an immature kerogen (from ~7 mbs) and a more deeply buried thermally mature shale sample (from ~1800 mbs) and chemically separated different fractions of the rock samples to obtain a maltene-free fraction and a maltene- and asphaltene-free fraction. Meslé et al. (2015) then did a series of culture

incubations wherein they incubated either bulk rock samples or each of the other fractions with a methanogenic consortium they previously enriched from immature kerogen and monitored these for methane production and microbial community composition using 454 pyrosequencing. Overall, the authors found that significantly more methane was produced from the deeper, more mature shale rock sample. This was expected as it would be characterized by a higher abundance of distinct hydrocarbons and TOC (29 mg/g) compared to the immature shallow shale (9 mg/g TOC) that would not have been heated enough to contain distinct hydrocarbons. Interestingly, though, the highest amount of methane that evolved from the immature shale-containing microcosms came from the maltene- and asphaltene-free fraction (compared to the bulk rock vs. maltene-free amendment), suggesting that solvent-insoluble OM entrained in kerogen rock could serve as a carbon source for methanogenic consortia. Members of the *Eubacteriaceae*, *Gracilibacteraceae*, and *Alcaligenaceae*, along with *Pseudomonas* spp. and methanogens, dominated the kerogen-amended incubations, which was distinct from taxa observed when acetate was added as an electron donor in separate incubations. The authors thus clearly demonstrated that kerogen OM could drive a syntrophic, shale-derived methanogenic community (Meslé et al. 2015). In a separate study conducted with metal-rich Kupferschiefer black shale (Poland), the authors found that strains isolated from the shale material could biodegrade a variety of hydrocarbons associated with the chloroform-extractable fraction of the shale under aerobic conditions (such as PAHs) (Matlakowska and Sklodowska 2011). This research group further demonstrated that these solvent-soluble kerogen components and other OM from the black shale could be degraded into a variety of identifiable alcohol and fatty acid metabolites, and many requisite oxygenases were also detected using a proteomic approach (Stasiuk et al. 2017). Collectively, the limited studies to date with oil shale kerogen from different locations have demonstrated the presence of indigenous microbial communities that appear to thrive on both the solvent-soluble and solvent-insoluble components of kerogen OM as carbon and energy sources.

As noted earlier, many shale deposits have undergone sufficient burial and heating to produce recognizable crude oil (shale oil) and gas (shale gas) that can be recovered using a combination of horizontal drilling and hydraulic fracturing ("fracking") techniques that allow deeply buried shale formations to release trapped oil or gas (Colosimo et al. 2016). In the USA and Canada, this recovery technique is applied widely in order to access oil and gas resources (e.g., from the Bakken, Barnett, Eagle Ford, Antrim, New Albany, Marcellus, Montney shale formations, etc.). In this procedure, water mixed with a variety of chemicals is injected downhole at extremely high pressure that cracks (fractures) the low-permeability shale, releasing hydrocarbons that can then be recovered. There has been a surge in investigations since 2010 regarding the microbial compositions related to these recovery operations in the interest of understanding both the ability of extant communities to produce biogas and to understand, in some cases, detrimental microbial processes such as souring and microbial corrosion that are emerging in some operations (Mouser et al. 2016; Colosimo et al. 2016). Souring, which refers to the generation of H_2S primarily through the activity of sulfate-reducing microorganisms within

reservoirs, is a highly undesirable process because H_2S is a highly toxic molecule that can affect the health and safety of workers and its presence in oil and gas devalues their quality (Gieg et al. 2011; Johnson et al. 2017). Mouser et al. (2016) recently overviewed the literature that describes the microbial community compositions associated with fracking operations. Notably, time course studies that examined the microbial composition of flowback water (water that returns to the surface with the released oil or gas) showed that initial microbial signatures that are distinct from the water used to create fractures were comprised primarily of halophilic or halotolerant *Gammaproteobacteria* such as *Marinobacter*, *Pseudomonas*, and *Acinetobacter* (Mohan et al. 2016; Cluff et al. 2014). With increasing time of flowback following fracking, the salinity typically increases, and microbial signatures indicate lower diversity with *Halanaerobium* and the methanogen *Methanohalophilus* being reported as dominant taxa in multiple shale oil and gas operations (Mouser et al. 2016; Lipus et al. 2017; An et al. 2017). Importantly, *Halanaerobium* can produce sulfide during metabolism, which has huge implications for souring and corrosion in fracturing operations (Liang et al. 2016a; Booker et al. 2017). While a detailed review of the microbes associated with hydraulic fracturing operations is beyond the scope of this chapter, identifying the microbial community compositions and putative activities associated with these recovery operations has yielded important information for managing potential problems such as souring (Daly et al. 2016; Mouser et al. 2016; Booker et al. 2017). Having baseline information regarding microbes and metabolic functions associated with oil shale kerogen deposits not only allows for a deeper understanding of their impact on the global carbon and oxygen cycles (Petsch et al. 2001) but can also help to inform recovery practices should these deposits ever be extensively exploited for energy.

7 Microbial Communities Associated with Biodegraded Oil Reservoirs

Within the last couple of decades, geochemical and microbiological studies have clearly demonstrated that deep subsurface crude oil reservoirs contain active and diverse microbial communities that contributed to the formation of heavy oil. Bastin et al. (1926) are credited with the first report of microorganisms existing in oilfields which was confirmed in the following decades (e.g., Zobell 1946; Magot et al. 2000). Much of the early work in identifying microorganisms associated with oilfields was done using cultivation techniques (Magot et al. 2000). However, the development and use of 16S rRNA gene sequencing (through cloning or next-generation sequencing, NGS) directly on environmental samples without cultivation have greatly expanded our knowledge on the kinds of microorganisms that are present in oil reservoirs. Even more, with the ability to now generate and assemble billions of DNA bases from different environments (including from oilfield samples), hundreds of bacterial and archaeal genomes are now available as blueprints for predicting metabolic potential and understanding any observed metabolism, such as oilfield souring (Hu et al. 2016). Importantly, extensive petrochemical and

geochemical measurements of hundreds of crude oils and reservoir rock properties have been key in interpreting microbial community compositions and functions within crude oil reservoirs. For example, the identification of known anaerobic hydrocarbon metabolites in biodegraded reservoirs links chemical analysis to known metabolic pathways and provides unequivocal evidence for in situ anaerobic hydrocarbon metabolism (Aitken et al. 2004; Gieg et al. 2010; Bian et al. 2015). Microbiological and geochemical data interpreted in concert have led to a greater understanding of factors that were likely in play over geological time transforming light crude oil to heavy crude oil.

Numerous recent articles (including reviews) have described microbial communities that inhabit heavy oilfields, the environmental controls on in situ crude oil biodegradation rates, and key principles associated with in situ biodegradation processes (e.g., Head et al. 2003, 2010, 2014; Larter et al. 2003, 2006; Röling et al. 2003; Magot 2005; Bennett et al. 2013; Varjani and Gnansounou 2017). The physical and geochemical controls on biodegradation include temperature, nutrient availability, salinity, electron donor/acceptor availability, and water availability (Head et al. 2003, 2010, 2014). Notably, as described above, extensive petrochemical analyses have shown that most biodegraded oil reservoirs have a temperature limit of ~80 °C (Wilhelms et al. 2001; Larter and Head 2014) and that high salinity levels ("paleopickling") can also potentially limit in situ crude oil biodegradation (Head et al. 2014). A combination of both high temperature and high salinity was shown to severely limit methanogenesis, a dominant process that led to the formation of biodegraded oil reservoirs (Head et al. 2014) (see below). Geochemical evidence has revealed that crude oil components within a heavy oil deposit are more depleted in an oil column as its proximity approaches the underlying water leg, paralleling an increase in oil viscosity (Larter and Head 2014, and references within). These data have suggested that the bulk of in situ oil biodegradation occurs primarily near the oil-water transition zone (OWTZ). Here, microbial communities benefit from an abundance of electron donors (hydrocarbons) in the oil leg and enhanced concentrations of nutrients (e.g., minerals dissolved from rock) that can help support hydrocarbon metabolism. Using qPCR analyses to quantify the 16S rRNA gene abundance (as a surrogate for total numbers of microorganisms) as a function of depth in a biodegraded reservoir, Bennett et al. (2013) showed that the highest 16S rRNA gene abundances were in the oil-leg and water-leg samples closest to the OWTZ, providing microbiological support for the geochemical and oil quality measurements.

Microorganisms spanning the range of biological redox reactions have been detected in oil reservoir fluids. These include aerobes, fermentative organisms, facultative aerobes, nitrate reducers, metal reducers, sulfur reducers, thiosulfate reducers, sulfate reducers, and methanogens (Magot 2005; Head et al. 2014; Varjani and Gnansounou 2017; Vigneron et al. 2017). In general, it has been difficult to determine whether identified microorganisms are truly indigenous to the deep subsurface crude oil reservoir habitat, because the majority of reservoir fluids that have been analyzed have come from reservoirs undergoing secondary recovery wherein water from outside sources (containing exogenous microbial inoculants)

are injected to facilitate oil recovery (Magot 2005). Studies that have analyzed samples from oilfields in primary production (not undergoing water-flooding), primarily from thermogenic fields, have revealed the presence of thermophilic microorganisms that are likely indigenous such as phylotypes classified as *Thermotoga*, *Geotoga*, *Thermoanaerobacter*, or *Thermococcus* (e.g., Grassia et al. 1996; Orphan et al. 2000; Vigneron et al. 2017). Further, isolates or enrichment cultures developed from oilfield fluids that grow or metabolize at temperatures of their requisite oilfields most likely represent the indigenous community members (L'Haridon et al. 1995; Magot 2005; Gieg et al. 2010; Head et al. 2014). Sierra-Garcia et al. (2017) recently examined the microbial composition (based on clone libraries) of a biodegraded and two non-biodegraded crude oil samples from hot Brazilian oilfields (e.g., 50–80 °C) that had not been water-flooded; thus any identified microorganisms were presumably associated with the reservoir environment. The authors found key differences in the oil samples: the non-degraded oil samples were less diverse and dominated primarily by *Gammaproteobacteria* (*Marinobacter* and *Marinobacterium* phylotypes), while the biodegraded oil was much more diverse, comprising members of the *Deferribacteraceae* (dominant), *Firmicutes*, *Thermotogae* (*Kosmotoga* and *Petrotoga*), and *Proteobacteria* (with *Gammaproteobacteria* representing only 1% of the clones). In a seminal study, Vigneron et al. (2017) used multiple molecular biology tools (such as quantitative PCR, 16S rRNA gene sequencing, metagenomics) to assess the microbial community composition and putative metabolic functions of what appears to be the most extensive series of samples taken from across a production life of a reservoir – ranging from non-water-flooded to extensively water-flooded areas. They found large shifts in the microbial community composition, with the non-water-flooded reservoir samples (with in situ temperatures ~75–80 °C) being dominated by strict anaerobic (and putative hydrocarbon-degrading) bacterial taxa affiliating with the *Thermotogales* and *Clostridiales*. With increasing amounts/history of water-flooding (which lowered the reservoir temperature to ~50 °C), the microbial community shifted to being dominated by phylotypes associated with the *Deferribacteres* and *Proteobacteria* having metabolic potentials dominated by nitrate reduction and sulfur compound oxidation. This study clearly demonstrates the impact of altering an oilfield for improved production on reservoir microbial communities (Vigneron et al. 2017).

Despite diverse taxa identified in various oilfield fluids, extensive geochemical evidence (such as gas cap methane and CO_2 indicating a biogenic isotopic signature) has strongly suggested that the major hydrocarbon biodegradation processes that transformed light crude oil to heavy crude-bearing reservoirs over geological time occurred under methanogenic conditions (Head et al., 2003, 2014). There has now been a multitude of reports using laboratory cultures showing that crude oil hydrocarbons can be converted to methane (e.g., Zengler et al. 1999; Townsend et al. 2003; Jones et al. 2008; Gieg et al. 2008, 2011), some of which were enriched from oilfield fluids (Gieg et al. 2010; Zhou et al. 2012; Berdugo-Clavijo and Gieg 2014; Liang et al. 2016b). This process is carried out by a syntrophic community of bacteria (such as members of the *Syntrophaceae*, *Firmicutes*, *Thermodesulfovibrio*,

Anaerolineaceae) that metabolize the hydrocarbons and convert these to methanogenic substrates for methanogens to use in a thermodynamically interdependent manner (Gieg et al. 2014). Though acetotrophic methanogens are present in many oil-degrading enrichment cultures, Jones et al. (2008) made the case based on thermodynamic and empirical measurements that crude oil degradation in situ more strongly involves H_2/CO_2-utilizing methanogens and coined the acronym MADCOR (*m*ethanogenic *a*lkane degradation *d*ominated by *C*O_2 *r*eduction).

8 Microbial Communities Associated with Bitumen and Tars

8.1 Oil Sands Bitumen

Bitumen deposits (such as those found in Alberta's oil sands region) as well as other ultra-heavy pits (e.g., La Brea tar pits in California) and asphalt lakes (such as Pitch Lake in Trinidad and Tobago) are characterized by highly biodegraded crude oil signatures enriched in heavy PAH, heterocyclic compounds, and asphaltenes that are highly heterogeneous in nature (Selucky et al. 1977). Though not as well characterized microbiologically compared to more conventional crude oil reservoir ecosystems, there are now several reports revealing that these ultra-heavy oil deposits do harbor microbial communities, and in some cases, putative metabolic functions of these communities have also been described.

Information to date in the peer-reviewed literature regarding microbial communities and metabolic functions associated with bitumen deposits has been primarily derived from studies within Canada's oil sands region. As described above, the Athabasca oil sands region located in northeastern Alberta, Canada resulted from the uplifting of deeper reservoirs making initially light crude oil conducive to extensive biodegradation over geological time. The bitumen deposits in this region are present at various depths, which determines how the bitumen can be recovered. At depths to 75 mbs, oil sands are surface-mined, while deeper bitumen is recovered using in situ heating technologies (Gates and Larter 2014). Notably, the Athabasca River and its tributaries in this region naturally flow through the oil sands deposits; thus natural bitumen-containing outcrops are ubiquitous along several riverbanks in the area (Wyndham and Costerton 1981a; Wong et al. 2015). With these water bodies, there is additional concern about the solubilization of some compounds found within bitumen such as naphthenic acids that have known toxic effects on many types of aquatic life (Li et al. 2017). As described below, microbial communities are present in all of these bituminous deposits (shallow and deep oil sands, riverbank outcrops, and bitumen-exposed river sediments). It should be noted that microbial communities present in the tailings ponds that hold waste solids and fluids following oil sands surface mining are not a focus of this chapter but are well described in a recent review (e.g., Foght et al. 2017). Though naphthenic acids are a non-hydrocarbon component of bitumen and other crude oils, the microbial ecology associated with these compounds in the environment (including in Canada's oil sands region) is the

focus of another chapter in this manual (Skeels and Whitby 2018) and will not be described here.

Early work by Wyndham and Costerton (1981a, b) used sediments collected from a variety of rivers near the Athabasca oil sands operations in order to investigate whether extant microbial communities were adapted to biodegrading PAHs, given their long-term exposure to bitumen. Using radiolabeled naphthalene and phenanthrene, they found that the sediment associated microbial communities did indeed have the ability to biodegrade PAHs under aerobic conditions (Wyndham and Costerton 1981a) and that bitumen-exposed river sediments biodegraded PAHs to a greater extent than sediments not exposed to bitumen. The authors further demonstrated that the indigenous microbes formed surface attachments (e.g., biofilms) to sands and associated bituminous compounds and they also isolated a number of bacterial strains that could biodegrade bitumen fractions (Wyndham and Costerton 1981b). Three decades later, following the development and widespread use of NGS (next generation sequencing), Yergeau et al. (2012) and Wong et al. (2015) evaluated river sediments and outcrops in the region for the presence of microbial communities using these molecular biology tools. In the interest of pinpointing taxa that may serve as bio-indicators for contamination in the oil sands region, Yergeau et al. (2012) collected sediment samples from locations along the Athabasca River and its tributaries and from oil sands tailings ponds and surveyed these for microbial community composition by 16S rRNA gene sequencing. They also conducted detailed hydrocarbon and naphthenic acid analysis to help interpret their results. The authors were able to clearly show that microbial community compositions in river sediment samples collected closer to sites of oil sands tailings ponds were more similar than those collected from a more distant location. Further, their analysis revealed several (at least ten) taxa that were positively correlated with the highest concentrations of PAHs and NAs in the samples (all members of the *Actinobacteria*, *Betaproteobacteria*, or *Bacteroidetes*), suggesting these as putative bio-indicators of contamination in the region (Yergeau et al. 2012). Wong et al. (2015) sampled bitumen outcrops found along the banks and within Athabasca River tributaries and assessed microbial community composition using 16S/18S rRNA gene sequencing (for bacteria, archaea, and fungi), genetic potential (using shotgun metagenomics), bitumen composition (using FTICR-MS analysis), and prospects for aerobic and anaerobic bitumen biodegradation. While some evidence for bitumen biotransformation was observed in aerobic incubations, none was apparent anaerobically, even though some known anaerobic phylotypes (such as methanogens) were detected in the community analyses. However, in accordance with changes in the nature of the bitumen in aerobic incubations, aerobes and fungi were most abundant in oil sands outcrops. Metagenomics analysis further revealed an abundance of genes encoding known bacterial aerobic hydrocarbon biodegradation enzymes (e.g., mono- and dioxygenases) and fungal cytochrome P_{450} oxidases (Wong et al. 2015); by comparison, no genomic evidence for anaerobic hydrocarbon-degrading enzymes was found. Also of interest is that some thermophilic taxa were detected in the NGS analyses which could be explained by field measurements showing that temperatures of these outcrops could reach as high as 60 °C on hot summer days

(e.g., when air temperatures are close to 30 °C). In all, the authors demonstrated that bitumen biodegradation in oil sands outcrops does occur, primarily under aerobic conditions.

To date, only two peer-reviewed studies have reported on the microbial community compositions entrained in deeper subsurface oil sands materials. Hubert et al. (2012) collected formation water associated with a "shallow" bitumen deposit (Suncor's Muskeg River Mine) that extended to 80 mbs, along with oil sands from an internal part of the mine and close to the OWTZ. The authors assessed the microbial composition of these materials using 16S rRNA gene analysis (via clone libraries and DGGE analysis) and intact polar lipids. In addition, chemical analysis of the formation waters and bitumen were conducted. Interestingly, they found that members of the class *Epsilonproteobacteria* dominated the formation water samples, within which the genera *Sulfuricurvum*, *Arcobacter*, and *Sulfurospirillum* were most prevalent (in decreasing abundance). Hydrocarbon measurements along a depth gradient within this bitumen deposit showed a depletion of dibenzothiophene close to the OWTZ where the *Sulfuricurvum* sequences were most abundant. This, along with previous reports that a *Sulfuricurvum* strain could metabolize S compounds, led the authors to postulate that this phylotype is a key sulfur heterocycle degrader in oil reservoirs (Hubert et al. 2012). The detection of a high abundance of *Epsilonproteobacteria* within these formation waters was congruent with findings from another low-temperature biodegraded reservoir in the region (Grabowski et al. 2005).

Deeper oil sands core samples were also recently analyzed using a metagenomics approach. An et al. (2013) obtained a 1 m core sample from an exploratory drill site of deep oil sands (298–299 mbs) located in Alberta's oil sands regions, divided these into 5 cm sections and conducted both 16S rRNA gene amplicon and shotgun sequencing of materials within the center of the core. Phylogenetic analysis revealed that substantial variation in taxa as a function of depth (e.g., communities were heterogeneous) and, surprisingly, a large portion of the identified taxa could be classified as aerobes rather than as anaerobes as would be predicted in subsurface oil reservoirs (Head et al. 2003; Larter et al. 2003, 2006). In addition, a number of aerobic "oxygenases" were detected in the deep oil sand metagenome (An et al. 2013). In other deep oil sands core samples, Ridley et al. (2017) also reported finding only "patchy" distribution of anaerobic taxa such as methanogens along a depth profile with the majority of sequences aligning with fungal or aerobic bacterial taxa. These results, along with a few other reports citing the identification of aerobic bacteria like *Pseudomonas* in methanogenic cultures (e.g., Guo et al. 2008; Berdugo-Clavijo and Gieg 2014), led Head et al. (2014) to suggest a variety of possible explanations for this observation including O_2 contamination of samples, the existence of a cryptic aerobic community (wherein O_2 is generated in metabolic processes that can then be used by aerobes), O_2 arising through meteoric waters, or that some aerobes/facultative aerobes (such as *Pseudomonas* spp.) can serve as syntrophic partners for methanogens. Reconciling these seemingly aerobic taxa in heavy oil reservoirs where other measurements strongly indicate a strictly anoxic environment requires much further study.

8.2 Tar Pits and Asphalt Lakes

The La Brea tar pits located in urban Los Angeles, California, USA, is a popular national landmark most well-known for trapping large animals from the geologic past. However, these tar pits represent an easily accessible example of a natural terrestrial surface seep wherein heavily biodegraded crude oil bubbles up from the subsurface. Kim and Crowley (2007) were the first to show that this asphalt-like material also harbors diverse microbial communities that are presumably slowly utilizing recalcitrant hydrocarbons within the pits, ultimately giving rise to methane bubbles that frequently discharge from the pits. They postulated that water pockets throughout the tar pits likely serve as the habitat for microbial communities, later proven to be the case by Meckenstock et al. (2014) who studied another asphalt-laden site (see below). Using 16S rRNA gene analysis (of 235 clones) of two different pit samples characterized by different chemical properties (e.g., pH and salinity), they found diverse members of Bacteria and Archaea (Kim and Crowley 2007). In the more saline pit, they detected a variety of halophilic Archaea (*Natronococcus* and *Natronobacterium*) not found in the less saline pit. Diverse bacterial taxa were detected, with *Gammaproteobacteria* dominating the clone libraries. Several new *Pseudomonas* genomovars were detected (e.g., known hydrocarbon-degrading bacteria), along with taxa classified as *Rubrobacteraceae*, a family of bacteria within the *Actinobacteria* phylum known to be highly resistant to ionizing radiation (Kim and Crowley 2007). Notably, the authors were also able to identify multiple mono- and dioxygenases associated with aerobic hydrocarbon biodegradation, several of which were novel based on a phylogenetic analysis. These results suggest that heavily biodegraded seep environments may be a source of new genes and enzymes (Kim and Crowley 2007). Belcher et al. (2012) subsequently isolated several strains from the La Brea tar pits and demonstrated that they could produce biosurfactants, which presumably aid in the solubilization and biodegradation of asphalt-like components.

Pitch Lake is another natural terrestrial ultra-heavy oil seep located in Trinidad and Tobago that has been recently assessed for its microbial community composition. This location has been deemed an asphalt lake, wherein the primary hydrocarbon components are in the form of asphaltenes, known to consist of sheets of polycyclic aromatic hydrocarbons intertwined with alkyl side chains and containing abundant N, S, and O atoms and heavy metals (Strausz et al. 1992). An analysis by Schulze-Makuch et al. (2011) revealed that gas bubbles produced from the lake consisted primarily of methane (73%) with minor amounts of ethane, propane, and butane. These authors also conducted a microbial survey and gene analysis on several samples collected from Pitch Lake. Using phospholipid fatty acid analysis, the authors found that Pitch Lake material harbored microorganisms ranging from 10^4 to 10^6 cells/g and that taxa included members of both the Bacteria and Archaea. Archaeal taxa included sequences affiliating with ANME (anaerobic methane-oxidizing archaea), *Thermoplasmatales* (members of which are known to be metal-respiring and/or thermoacidophiles), along with some novel lineages. Bacterial taxa included some commonly known hydrocarbon degraders, such as pseudomonads,

along with known sulfur compound-utilizing bacteria. Overall, the microbial community profile indicated a primarily anaerobic (methanogenic) signature, and given the comparatively low water content of this environment, the authors proposed it to be an excellent analog for the hydrocarbon pools that exist on Titan (Saturn's moon) (Schulze-Makuch et al. 2011). Meckenstock et al. (2014) went on to show that methanogenic microbial communities and activities in Pitch Lake were associated with water droplets dispersed throughout the asphalt – collectively comprising a 13% water content which was more than adequate for microbial life and activity. The results from this surface asphalt deposit were extrapolated to the deeper subsurface environment of an oil reservoir to postulate that microbial activity contributing to crude oil biodegradation would not be limited to the OWTZ but can also potentially occur in small water droplets dispersed throughout an entire oil leg (Meckenstock et al. 2014). Kryachko et al. (2012) clearly showed that different microbial taxa were associated with the crude oil versus produced water phases of an oil/water sample collected from a low-temperature oilfield, further emphasizing that microbes can exist (presumably in micro-water droplets) within crude oil itself.

9 Research Needs

As overviewed here, the majority of our understanding of the microbial communities that inhabit oil-bearing reservoirs comes from studies examining biodegraded conventional crude oil reservoirs rather than "pre-oil" shale or ultra-heavy bitumen/asphalt deposits. Though a few additional studies of these latter deposits have provided additional microbiological information in the past few years, much more research is required to understand some of the recent observations. For example, in Alberta's deep oil sands region, a system where geochemical signatures have overwhelmingly demonstrated an anoxic (methanogenic) system, why have so many "aerobic" taxa recently been detected using NGS? Is this an artifact, or is this observation providing clues to new metabolisms that we do not yet appreciate? Though substantial strides have been made in understanding the environmental factors that influence the extent of crude oil biodegradation within reservoirs, our understanding is far from complete (Head et al. 2014). Of the environments discussed in this chapter, oil shale deposits have received the least attention as only a handful of oil shale formations have been examined microbiologically. A microbiological assessment of other geographically distinct oil shale deposits (e.g., from the Eocene Green River formation) is still needed, which will vastly increase our understanding of life potentially eking out a living in these poorly characterized geobiospheres.

Understanding the metabolic potentials of microbial communities inhabiting these varied deposits has far-reaching implications toward biotechnology development (Bachmann et al. 2014). For example, the fact that crude oil and shale OM can be biodegraded under methanogenic conditions has led to the prospects for enhanced energy recovery as methane (rather than oil, and a cleaner burning energy source) from marginal reservoirs (Gray et al. 2010) and from kerogen

deposits (Martini et al. 1996; Meslé et al. 2015). Work done decades ago in using microorganisms to enhance porosity in the Green River oil shale formation showed promising results (e.g., Findley et al. 1974; Meyer and Yen 1976). With a better understanding of extant microbial communities in oil shale, can this technology be revisited to help recovery oil shale energy in a more economical manner? Finally, given the global push toward reducing greenhouse gas levels, the prospect has been raised that instead of recovering fossil energy to combust, perhaps the energy entrained within challenging deposits (e.g., deep bitumen reserves) can remain in the subsurface. Instead, the hydrocarbon-degrading metabolism of extant microorganisms can be exploited in situ to drive the generation of electricity, a much cleaner energy source (Larter and Head 2014; Head and Gray 2016). Exploiting in situ microorganisms to increase oil recovery has been considered for decades (Siegert et al. 2014); thus such a scheme has great possibility. Clearly, a much-needed deeper understanding of the metabolic potential of microbial communities within diverse hydrocarbon resource environments is key to developing such new energy technologies.

References

Aitken CM, Jones DM, Larter SR (2004) Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs. Nature 431:291–294

An D, Caffrey SM, Soh J, Agrawal A, Brown D, Budwill K, Dong X, Dunfield PF, Foght J, Gieg LM, Hallam SJ, Hanson NW, He Z, Jack TR, Klassen J, Konwar KM, Kuatsjah E, Li C, Larter S, Leopatra V, Nesbø CL, Oldenburg T, Pagé AP, Ramos-Padron E, Rochman FF, Saidi-Mehrabad A, Sensen CW, Sipahimalani P, Song YC, Wilson S, Wolbring G, Wong ML, Voordouw G (2013) Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. Environ Sci Technol 47:10708–10717

An BA, Shen Y, Voordouw G (2017) Control of sulfide production in high salinity Bakken shale oil reservoirs by halophilic bacteria reducing nitrate to nitrite. Front Microbiol 8:1164

Bachmann RT, Johnson AC, Edyvean RJG (2014) Biotechnology in the petroleum industry: an overview. Int Biodeterior Biodegrad 86:225–237

Bastin ES, Greer FE, Merritt CA, Moulton G (1926) The presence of sulphate-reducing bacteria in oil field waters. Science 63:1865–1883

Belcher RW, Huynh KV, Hoang TV, Crowley DE (2012) Isolation of biosurfactant-producing bacteria from the Rancho La Brea tar pits. World J Microbiol Biotechnol 28:3261–3267

Bennett B, Adams JJ, Gray ND, Sherry A, Oldenburg TBP, Huang H, Larter SR, Head IM (2013) The controls on the composition of biodegraded oils in the deep subsurface – part 3. The impact of microorganism distribution on petroleum geochemical gradients in biodegraded petroleum reservoirs. Org Geochem 56:94–105

Berdugo-Clavijo C, Gieg LM (2014) Conversion of crude oil to methane by a microbial consortium enriched from oil reservoir production waters. Front Microbiol 5:197

Bian XY, Mbadinga SM, Liu YF, Yang SZ, Liu JF, Ye RQ, Gu JD, Mu BZ (2015) Insights into the anaerobic biodegradation pathway of *n*-alkanes in oil reservoirs by detection of signature metabolites. Sci Rep 5:9801

Booker AE, Borton MA, Daly RA, Welch SA, Nicora CD, Hoyt DW, Wilson T, Purvine SO, Wolfe RA, Sharma S, Mouser PJ, Cole DR, Lipton MS, Wrighton KC, Wilkins MJ (2017) Sulfide generation by dominant *Halanaerobium* microorganisms in hydraulically fractured shales. mSphere 2(4). pii: e00257–17

Cluff MA, Hartsock A, MacRae JD, Carter K, Mouser PJ (2014) Temporal changes in microbial ecology and geochemistry in produced water from hydraulically fractured Marcellus shale gas wells. Environ Sci Technol 48:6508–6517

Colosimo F, Thomas R, Lloyd JR, Taylor KG, Boothman C, Smith AD, Lord R, Kalin RM (2016) Biogenic methane in shale gas and coal bed methane: a review of current knowledge and gaps. Int J Coal Geol 165:106–120

Colwell FS, D'Hondt S (2013) Nature and extent of the deep biosphere. Rev Mineral Geochem 75:547–574

Daly RA, Borton MA, Wilkins MJ, Hoyt DW, Kountz DJ, Wolfe RA, Welch SA, Marcus DN, Trexler RV, MacRae JD, Krzycki JA, Cole DR, Mouser PJ, Wrighton KC (2016) Microbial metabolisms in a 2.5-km-deep ecosystem created by hydraulic fracturing in shales. Nat Microbiol 5:16146

Dyni JR (2005) Geology and resources of some world oil-shale deposits. Scientific investigations report 2005–5294, Department of the Interior, US Geological Survey

Findley MD, Appleman MD, Yen TF (1974) Degradation of oil shale by sulfur-oxidizing bacteria. Appl Microbiol 28:460–464

Foght J (2010) Microbial communities in oil shales, biodegraded and heavy oil reservoirs, and bitumen deposits. In: Timmis KN, McGenity TJ (eds) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2161–2172

Foght J, Gieg LM, Siddique T (2017) Microbiology of oil sands tailings: past, present, future. FEMS Microbiol Ecol 3(5). https://doi.org/10.1093/femsec/fix034

Gates ID, Larter SR (2014) Energy efficiency and emissions intensity of SAGD. Fuel 115:706–713

Gieg LM, Duncan KE, Suflita JM (2008) Bioenergy production via microbial conversion of residual oil to natural gas. Appl Environ Microbiol 74:3022–3029

Gieg LM, Davidova IA, Duncan KE, Suflita JM (2010) Methanogenesis, sulfate reduction and crude oil biodegradation in hot Alaskan oilfields. Environ Microbiol 12:3074–3086

Gieg LM, Jack TR, Foght JM (2011) Biological souring and mitigation in oil reservoirs. Appl Microbiol Biotechnol 92:263–282

Gieg LM, Fowler SJ, Berdugo-Clavijo C (2014) Syntrophic biodegradation of hydrocarbon contaminants. Curr Opin Biotechnol 27:21–29

Grabowski A, Nercessian O, Fayolle F, Blanchet D, Jeanthon C (2005) Microbial diversity in production waters of a low-temperature biodegraded oil reservoir. FEMS Microbiol Ecol 54:427–443

Grassia GS, McLean KM, Glénat P, Bauld J, Sheehy AJ (1996) A systematic survey for thermophilic fermentative bacteria and archaea in high temperature petroleum reservoirs. FEMS Microbiol Ecol 21:47–58

Gray ND, Sherry A, Hubert C, Dolfing J, Head IM (2010) Methanogenic degradation of petroleum hydrocarbons in subsurface environments remediation, heavy oil formation, and energy recovery. Adv Appl Microbiol 72:137–161

Gray ND, Sherry A, Grant RJ, Rowan AK, Hubert CR, Callbeck CM, Aitken CM, Jones DM, Adams JJ, Larter SR, Head IM (2011) The quantitative significance of *Syntrophaceae* and syntrophic partnerships in methanogenic degradation of crude oil alkanes. Environ Microbiol 13:2957–2975

Guo L, Li XM, Bo X, Yang Q, Zeng GM, Liao DX, Liu JJ (2008) Impacts of sterilization, microwave and ultrasonication pretreatment on hydrogen producing using waste sludge. Bioresour Technol 99:3651–3658

Head IM, Gray ND (2016) Microbial biotechnology 2020; microbiology of fossil fuel resources. Microb Biotechnol 9:626–634

Head IM, Jones DM, Larter SR (2003) Biological activity in the deep subsurface and the origin of heavy oil. Nature 426:244–352

Head IM, Larter SR, Gray ND, Sherry A, Adams JJ, Aitken CM et al (2010) Hydrocarbon degradation in petroleum reservoirs. In: Timmis KN, McGenity TJ (eds) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 3097–3109

Head IM, Gray ND, Larter SR (2014) Life in the slow lane; biogeochemistry of biodegraded petroleum containing reservoirs and implications for energy recovery and carbon management. Front Microbiol 5:566

Hu P, Tom L, Singh A, Thomas BC, Baker BJ, Piceno YM, Andersen GL, Banfield JF (2016) Genome-resolved metagenomic analysis reveals roles for candidate phyla and other microbial community members in biogeochemical transformations in oil reservoirs. mBio 7: e01669–e01615

Hubert CRJ, Oldenburg TBP, Fustic M, Gray ND, Larter SR, Penn K, Rowan AK, Seshadri R, Sherry A, Swainsbury R, Voordouw G, Voordouw JK, Head IM (2012) Massive dominance of *Epsilonproteobacteria* in formation waters from a Canadian oil sands reservoir containing severely biodegraded oil. Environ Microbiol 14:387–404

Hunt JM (1996) Petroleum geochemistry and geology, 2nd edn. W.H. Freeman and Company, New York

Johnson RC, Mercier TJ, Brown ME, Pantea, MP, Self JG (2010) An assessment of in-place oil shale resources in the Green River formation, Piceance Basin. U.S. Geological Survey Digital Data Series DDS–69–Y, Colorado, 187 pp

Johnson RJ, Folwell BD, Wirekoh A, Frenzel M, Skovhus TL (2017) Reservoir souring – latest developments for application and mitigation. J Biotechnol 256:57–67

Jones DM, Head IM, Gray ND, Adams JJ, Rowan AK, Aitken CM, Bennett B, Huang H, Brown A, Bowler BF, Oldenburg T, Erdmann M, Larter SR (2008) Crude oil biodegradation via methanogenesis in subsurface petroleum reservoirs. Nature 451:176–180

Kim J-S, Crowley DE (2007) Microbial diversity in natural asphalts of the Rancho La Brea tar pits. Appl Environ Microbiol 73:4579–4591

Kryachko Y, Dong X, Sensen CW, Voordouw G (2012) Compositions of microbial communities associated with oil and water in a mesothermic oil field. Antonie Van Leeuwenhoek 101:493–506

L'Haridon S, Reysenbach A-L, Glénat P, Prieur D, Jeanthon C (1995) Hot subterranean biosphere in a continental oil reservoir. Nature 377:223–224

Larter SR, Head IM (2014) Oil sands and heavy oil: origin and exploitation. Elements 10:277–284

Larter SR, Wilhelms A, Head I, Koopmans M, Aplin A, Di Primio R, Zwach C, Erdmann M, Telnaes N (2003) The controls on the composition of biodegraded oils in the deep subsurface – part 1: biodegradation rates in petroleum reservoirs. Org Geochem 34:601–613

Larter S, Huang H, Adams J, Bennett B, Jokanola O, Oldenburg T, Jones M, Head I, Riediger C, Fowler M (2006) The controls on the composition of biodegraded oil in the deep subsurface: part II – geological controls on subsurface biodegradation fluxes and contraints on reservoir-fluid property prediction. AAPG Bull 90:921–938

Larter S, Huang H, Adams J, Bennett B, Snowdon LR (2012) A practical scale for use in reservoir geochemical studies of biodegraded oils. Org Geochem 45:66–76

Li C, Fub L, Stafford J, Belosevic GE-DM (2017) The toxicity of oil sands process-affected water (OSPW): a critical review. Sci Total Environ 601–602:1785–1802

Liang R, Davidova IA, Marks CR, Stamps BW, Harriman BH, Stevenson BS, Duncan KE, Suflita JM (2016a) Metabolic capability of a predominant *Halanaerobium* sp. in hydraulically fractured gas well and its implication in pipeline corrosion. Front Microbiol 7:988

Liang B, Wang LY, Zhou Z, Mbadinga SM, Zhou L, Liu JF, Yang SZ, Gu JD, Mu BZ (2016b) High frequency of *Thermodesulfovibrio* spp. and *Anaerolineaceae* in association with *Methanoculleus* spp. in a long-term incubation of *n*-alkanes-degrading methanogenic enrichment culture. Front Microbiol 7:1431

Lipus D, Vikram A, Ross D, Bain D, Gulliver D, Hammack R, Bibby K (2017) Predominance and metabolic potential of *Halanaerobium* spp. in produced water from hydraulically fractured Marcellus shale wells. Appl Environ Microbiol 83(8). pii: e02659-16

Magot M (2005) Indigenous microbial communities in oil fields. In: Ollivier B, Magot M (eds) Petroleum microbiology. ASM Press, Washington, pp 21–33

Magot M, Ollivier B, Patel BK (2000) Microbiology of petroleum reservoirs. Antonie Van Leeuwenhoek 77:103–116

Martini AM, Budai JM, Walter LM, Schoell M (1996) Microbial generation of economic accumulations of methane within a shallow organic-rich shale. Nature 383:155–158

Martini AM, Walter LM, McIntosh JC (2008) Identification of microbial and thermogenic gas components from upper Devonian Antrim black shale cores, Illinois and Michigan basins. Am Assoc Pet Geol Bull 92:327–339

Matlakowska R, Sklodowska A (2011) Biodegradation of Kupferschiefer black shale organic matter (Fore-Sudetic monocline, Poland) by indigenous microorganisms. Chemosphere 83:1255–1261

McIntosh JC, Walter LM, Martini AM (2002) Pleistocene recharge to midcontinent basins: effects on salinity structure and microbial gas generation. Geochim Cosmochim Acta 66:1681–1700

Meckenstock RU, von Netzer F, Stumpp C, Lueders T, Himmelberg AM, Hertkorn N, Schmitt-Kopplin P, Harir M, Hosein R, Haque S, Schulze-Makuch D (2014) Oil biodegradation. Water droplets in oil are microhabitats for microbial life. Science 345:673–676

Meslé M, Périot C, Dromart G, Oger PM (2012) Biostimulation to identify microbial communities involved in methane generation in shallow kerogen-rich shales. J Appl Microbiol 114:55–70

Meslé M, Dromart G, Haeseler F, Oger PM (2015) Classes of organic molecules targeted by a methanogenic microbial consortium grown on sedimentary rocks of various maturities. Front Microbiol 6:589

Meyer WC, Yen TF (1976) Enhanced dissolution of oil shale by bioleaching with Thiobacilli. Appl Environ Microbiol 32:610–616

Mohan AM, Bibby KJ, Lipus D, Hammack RW, Gregory KB (2016) The functional potential of microbial communities in hydraulic fracturing source water and produced water from natural gas extraction characterized by metagenomic sequencing. PLoS One 9:e107682

Mouser PJ, Borton M, Darrah TH, Hartsock A, Wrighton KC (2016) Hydraulic fracturing offers view of microbial life in the deep terrestrial subsurface. FEMS Microbiol Ecol 92(11). pii: fiw166

Orphan VJ, Taylor LT, Hafenbradl D, Delong EF (2000) Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. Appl Environ Microbiol 66:700–711

Peters KE, Moldowan JM (1993) The biomarker guide. Prentice Hall, New York

Petsch ST, Eglinton TI, Edwards KJ (2001) ^{14}C-dead living biomass: evidence for microbial assimilation of ancient organic carbon during shale weathering. Science 292:1127–1131

Petsch ST, Edwards KJ, Eglinton TI (2003) Abundance, distribution and δ^{13}C analysis of microbial phospholipid-derived fatty acids in a black shale weathering profile. Org Geochem 34:731–743

Pfister RM, Krieger E, Ridge J (1991) Microbial presence and potential in eastern oil shale. Resour Conserv Recycl 5:231–243

Ridley D, An D, Stancliffe S, Voordouw G (2017) Patchy distribution of methanogenic archaea and hydrocarbon-degrading fungi in northern Alberta oil sands deposits. Abstract, ISMOS-6, San Diego, 6–9 June

Roadifer RE (1987) Size distributions of the world's largest known oil and tar accumulations. In: Meyer F (ed) Exploration for heavy crude oil and natural bitumen. AAPG, Tulsa, pp 3–23

Röling WFM, Head IM, Larter SR (2003) The microbiology of hydrocarbon degradation in subsurface petroleum reservoirs: perspectives and prospects. Res Microbiol 154:321–328

Schlegel ME, McIntosh JC, Petsch ST, Orem WH, Jones EJP, Martini AM (2013) Extents and limits of biodegradation by *in situ* methanogenic consortia in shale and formation fluids. Appl Geochem 28:172–184

Schulze-Makuch D, Haque S, Resendes de Sousa Antonio M, Ali D, Hosein R, Song YC, Yang J, Zaikova E, Beckles DM, Guinan E, Lehto HJ, Hallam SJ (2011) Microbial life in a liquid asphalt desert. Astrobiology 11:241–258

Selucky ML, Chu Y, Ruo T, Strausz OP (1977) Chemical composition of Athabasca bitumen. Fuel 56:369–381

Siegert M, Sitte J, Galushko A, Kruger M (2014) Starting up microbial enhanced oil recovery. Adv Biochem Eng Biotechnol 142:1–94

Sierra-Garcia IN, Dallagnezze BM, Santos VP, Chaves MR, Capilla R, Santos Neto EV, Gray N, Oliveira VM (2017) Microbial diversity in degraded and non-degraded petroleum samples and comparison across oil reservoirs and local and global scales. Extremophiles 21:211–229

Skeels K, Whitby CM (2018) Microbial ecology of naphthenic acid (NA) degradation. In: McGenity, TJ (ed) Handbook of hydrocarbon and lipid microbiology, Springer, Berlin, in press

Stasiuk R, Włodarczyk A, Karcz P, Janas M, Skłodowska A, Matlakowska R (2017) Bacterial weathering of fossil organic matter and organic carbon mobilization from subterrestrial Kupfreschiefer black shale: long-term laboratory studies. Environ Microbiol Rep 9:459–466

Strausz OP, Mojelsky TW, Lown EM (1992) The molecular structure of asphaltene: an unfolding story. Fuel 71:1355–1363

Townsend GT, Prince RC, Suflita JM (2003) Anaerobic oxidation of crude oil hydrocarbons by the resident microorganisms of a contaminated anoxic aquifer. Environ Sci Technol 37:5213–5218

US Geological Survey (2017) https://energy.usgs.gov/OilGas/UnconventionalOilGas/OilShale.aspx#3894238-overview. Accessed 1 Oct

Utah Geological Survey (2017) https://geology.utah.gov/map-pub/survey-notes/oil-shale-vs-shale-oil-whats-the-difference/. Accessed 1 Oct

Vandenbroucke M, Largeau C (2007) Kerogen origin, evolution and structure. Org Geochem 38:719–833

Varjani SJ, Gnansounou E (2017) Microbial dynamics in petroleum oilfields and their relationship with physiological properties of petroleum oil reservoirs. Bioresour Technol 245:1258–1265.

Vigneron A, Alsop EB, Lomans BP, Kyrpides NC, Head IM, Tsesmetzis N (2017) Succession in the petroleum reservoir microbiome through and oil field production life cycle. ISME J 11:2141–2154

Wilhelms A, Larter SR, Head IM, Farrimond P, DiPrimio R, Zwach C (2001) Biodegradation of oil in uplifted basins prevented by deep-burial sterilization. Nature 411:1034–1037

Wong ML, An D, Caffrey SM, Soh J, Dong X, Sensen CW, Oldenburg TBP, Larter SR, Voordouw G (2015) Roles of thermophiles and fungi in bitumen degradation in mostly cold oil sands outcrops. Appl Environ Microbiol 81:6825–6838

Wyndham RC, Costerton JW (1981a) Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms with the Athabasca oil sands deposit. Appl Environ Microbiol 41:783–790

Wyndham RC, Costerton JW (1981b) In vitro microbial degradation of bituminous hydrocarbons and in situ colonization of bitumen surfaces within the Athabasca oil sands deposit. Appl Environ Microbiol 41:791–800

Yergeau E, Lawrence JR, Sanschagrin S, Waiser MJ, Korber DR, Greer CW (2012) Next-generation sequencing of microbial communities in the Athabasca River and its tributaries in relation to oil sands mining activities. Appl Environ Microbiol 78:7626–7637

Zengler K, Richnow HH, Rosselló-Mora R, Michaelis W, Widdel F (1999) Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 6750:266–269

Zhou S, Huang H, Yuming L (2008) Biodegradation and origin of oil sands in the Western Canadian sedimentary basin. Pet Sci 5:87–94

Zhou L, Li K-P, Mbadinga SM, Yang S-Z, Gu J-D, Mu B-Z (2012) Analyses of *n*-alkanes degrading community dynamics of a high-temperature methanogenic consortium enriched from production water of a petroleum reservoir by a combination of molecular techniques. Exotoxicology 21:1680–1691

ZoBell CE (1946) Action of microorganisms on hydrocarbons. Bacteriol Rev 10:1–49

Mechanisms and Monitoring of Oil Reservoir Souring Control by Nitrate or Perchlorate Injection

12

Hans K. Carlson and Casey R. J. Hubert

Contents

Abstract

Oil reservoir souring is the production of hydrogen sulfide by sulfate-reducing microorganisms (SRM) in oil fields. Anaerobic respiration of sulfate is supported by various electron donors in petroleum reservoir ecosystems. Nitrate addition results in souring control by stimulating dissimilatory nitrate-

H. K. Carlson
Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Lab, Berkeley, CA, USA
e-mail: carlsonh@berkeley.edu

C. R. J. Hubert (✉)
Department of Biological Sciences, University of Calgary, Calgary, AB, Canada
e-mail: chubert@ucalgary.ca

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_17

reducing microorganisms (NRM) that directly or indirectly utilize petroleum-derived SRM electron donors. The oxidative capacity of nitrate for this process depends on NRM physiology and whether nitrate is metabolized to fully reduced end products or is partially reduced to nitrite. Production of nitrite is beneficial because it inhibits SRM. In laboratory tests, similar to nitrate, perchlorate also results in inhibition of microbial sulfate reduction by stimulating dissimilatory perchlorate-reducing microorganisms (PRM). The intermediates of perchlorate respiration include the potent oxidants, chlorate, chlorite, and dioxygen, which, like nitrite, are also highly inhibitory of SRM. The two approaches to souring control have interesting similarities and differences with respect to mode of action, and we discuss ways in which they could have beneficial synergistic interactions in a co-treatment approach to souring control. Other oxyanion inhibitors of souring are an interesting area of future research, and we summarize data on their modes of action and impact on different microbial subpopulations. Oil companies use various microbiological surveillance tools to monitor the success of nitrate injection or other souring control strategies. SRM surveillance traditionally relies on cultivation-based testing but in recent years has expanded to include cultivation-independent molecular and isotopic methods for detection and quantification of both harmful and beneficial oil reservoir microbes.

1 Introduction

The petroleum industry's growing awareness of microbes and microbiology is due in part to the detrimental effects caused by anaerobic sulfate-reducing microorganisms (SRM). Reservoir souring is caused by microbial reduction of sulfate to sulfide by sulfate-reducing bacteria or archaea in the presence of appropriate nutrients, substrates, or conditions that get introduced during oil recovery operations. For example, seawater injection for production pressure maintenance both introduces sulfate and alters the formation temperature near the injection well, creating conditions permissive for SRM. Negative consequences of souring are many and include health and safety concerns (H_2S is a deadly gas), increased sulfur content in produced oil and gas, and SRM-influenced corrosion. Different souring control strategies exist. Sulfate removal from injection water by nanomembrane filtration has been demonstrated; however deployment is costly, technically challenging, and hence not widespread (Alkindi et al. 2013). More common are physical or chemical removal of H_2S post production (Jensen and Webb 1995) and the application of biocides to kill or inhibit reservoir SRM (Telang et al. 1998; Greene et al. 2006). Oil companies have widely used nitrate injection technology for souring control (Torsvik and Sunde 2005), and perchlorate treatment is an alternative approach that is gaining recognition and is likely to be tested in the field soon (Coates 2014; Carlson et al. 2014). Adding nitrate or perchlorate stimulates dissimilatory nitrate-reducing microorganisms (NRM) or dissimilatory perchlorate-reducing microorganisms (PRM), respectively, which can prevent or reverse the souring reactions

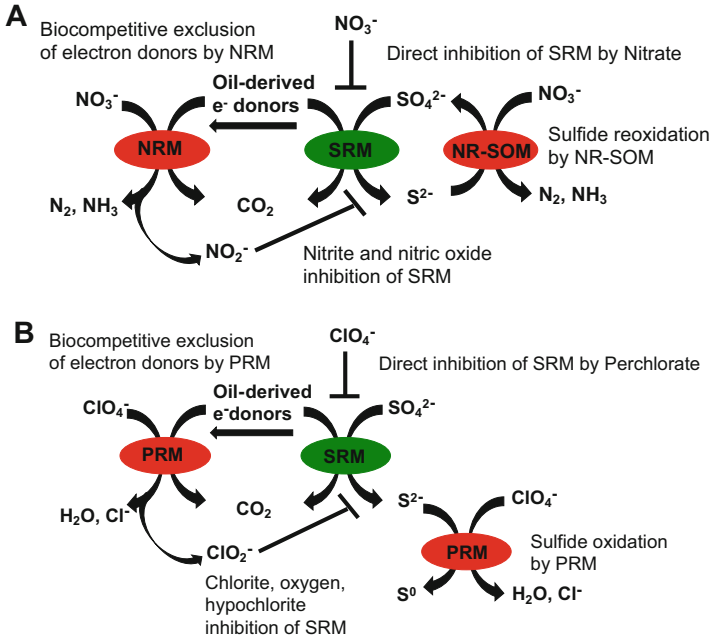

Fig. 1 (**a**) Souring control following nitrate injection can be achieved via biocompetitive exclusion of SRM electron donors by chemoorganotrophic NRM, or via direct oxidation of sulfide by chemolithotrophic sulfide-oxidizing NRM, as described in the text. Nitrite production by NRM can also inhibit SRM. At sufficiently high concentrations, nitrate can competitively inhibit sulfate reduction by SRM. (**b**) Souring control following perchlorate injection can be achieved via biocompetitive exclusion of SRM by chemoorganotrophic PRM. Sulfide is oxidized largely to elemental sulfur by PRM. Reactive chlorine and oxygen species produced by PRM are potent inhibitors of SRM. At sufficiently high concentrations, perchlorate can competitively inhibit sulfate reduction by SRM

catalyzed by SRM (Fig. 1). The two approaches share some similarities, but there are also important differences as the physiology and ecology of the respiratory processes are distinct. Managing reservoir microbial communities in this way is the focus of the present chapter.

Oil reservoirs represent complex microbial ecosystems featuring various interactions between different groups of anaerobes. These reduced environments are rich in electron donors but contain relatively few terminal electron acceptors. In this context, methanogenic degradation of petroleum hydrocarbons over long timescales is catalyzed in situ by consortia of reservoir microbes (Head et al. 2003). Secondary oil production (i.e., water injection to maintain in situ reservoir pressure and provide a water sweep of oil through the reservoir) can alter the microbial ecosystem dramatically by introducing oxidants – particularly sulfate when seawater is injected at offshore operations. Subsequent nitrate or perchlorate injection for souring control can affect the microbial ecology even further, resulting in interconnected anaerobic carbon, sulfur, nitrogen, and/or chlorine cycling. Understanding these cycles and

the interactions between SRM and NRM/PRM is of critical importance for smart management of reservoir souring problems.

The importance of reservoir souring is reflected by an increasing body of primary scientific literature, much of which is cited in several good reviews of souring and souring control (Eckford and Fedorak 2004; Birkeland 2005; Thrasher and Vance 2005; Torsvik and Sunde 2005; Grigoryan and Voordouw 2008; Ollivier et al. 2009; Gieg et al. 2011). The current chapter will focus specifically on aspects of SRM, NRM, and PRM physiology that are relevant to reservoir souring and souring control (Sects. 2 and 3, respectively). Different strategies oil companies use for monitoring microorganisms in oil-field settings will also be discussed (Sect. 5).

2 Sulfate Reduction in Oil Reservoirs

Souring is a likely outcome following seawater injection into oil reservoirs, which brings the terminal electron acceptor sulfate into contact with various electron donors in the organic-rich petroleum hydrocarbon environment. Essential nutrients (N, P, etc.) may be present both in injected water and in the reservoir. Microorganisms are widely believed to be indigenous to oil reservoirs, but they may also be introduced with the injected water. Oil-field SRM detected and characterized to date are physiologically and phylogenetically diverse, ranging from mesophilic *Deltaproteobacteria* to hyperthermophilic archaea to spore-forming mesophilic, thermophilic, and halophilic *Firmicutes* (Ollivier et al. 2009; Gittel et al. 2009; Aüllo et al. 2013; Vigneron et al. 2017). The physiology of oil-field SRM (e.g.,temperature and substrate range) can provide clues as to whether they are indigenous to a given reservoir habitat or introduced (Magot 2005). The chief concern here however is the production of sulfide, which is problematic for the oil industry regardless of the provenance of the culprit SRM.

2.1 Electron Donors in Oil Reservoir Ecosystems

Electron donors for sulfate-reducing microorganisms in anoxic environments are often organic acids, alcohols, and hydrogen that may be by-products of fermentative degradation of complex organic compounds. Organic acids, alcohols, and hydrogen are utilized by several characterized SRM from oil-field environments (Birkeland 2005; Grigoryan and Voordouw 2008), and organic acid anions are detectable in oil reservoir fluids (e.g., Barth 1991). Other potential electron donors in oil reservoirs are obviously petroleum hydrocarbons. SRM that oxidize alkanes, cycloalkanes, and aromatic compounds anaerobically have been isolated from various environments (Widdel et al. 2009); however few of the SRM detected in or isolated from oil fields described so far share this phenotype (Harms et al. 1999; Magot 2005). Although future efforts may uncover more oil-field isolates that couple hydrocarbon oxidation to sulfate reduction, the paucity of examples to date questions whether in situ reservoir souring is driven directly by hydrocarbons or rather by other electron donors.

Alternatively, SRM could be indigenous reservoir inhabitants that have a different lifestyle prior to industrial oil recovery activities. Anaerobic biodegradation of oil in situ involves microbial consortia that degrade hydrocarbons into acetate and hydrogen that are consumed by methanogens (Zengler et al. 1999; Head et al. 2003; Jones et al. 2008). When sulfate is scarce or absent, some SRM carry out fermentative metabolism and can serve as syntrophic partners for methanogens (Bryant et al. 1977; Muyzer and Stams 2008). Such organisms may switch from fermentation to sulfate reduction following injection of sulfate-rich seawater into an oil reservoir. A related possibility is that introduction of sulfate to a previously methanogenic system results in the competitive exclusion of methanogens by acetate- or hydrogen-oxidizing SRM. In this scenario reservoir souring would be driven directly by small organic acids and hydrogen produced by ongoing biodegradation of petroleum hydrocarbons; i.e., the early steps in oil degradation pathways would not change, but these processes are enabled by end members consuming acetate and hydrogen via sulfate reduction rather than methanogenesis.

2.2 Limiting Factors that Inform Souring Control Strategies

The extent of reservoir souring in the presence of abundant sulfate depends on the amount of electron donors and trace nutrients present to support the growth of SRM. Souring may also be limited by the presence of toxic or inhibitory compounds in petroleum that interfere with SRM metabolism (Torsvik and Sunde 2005), and temperature, salinity, pH, and mineral scavenging must also be considered (Johnson et al. 2017; Pannekens et al. 2019). These limiting factors form the basis for industrial strategies to combat SRM activity. Traditionally the introduction of toxic biocides has been used to curb microbial growth, inhibiting SRM in particular. Biocides are also toxic for humans and marine life (Greene et al. 2006). Biocide effectiveness is dependent on proper dosing regimens and the degree to which target microbes develop biocide resistance (Sanders and Sturman 2005). Other oxyanion treatments, such as molybdate, have also been long considered as selective inhibitors of sulfate reduction (Postgate 1952), although their use has largely been limited to ecological studies (Oremland and Capone 1988). Nitrate injection technology is currently the most widely used approach in the oil industry to control souring and is primarily based on a strategy to deplete SRM electron donors via nitrate reduction instead of sulfate reduction. Perchlorate injection is a newer approach to souring control that is based on similar mechanisms. Both nitrate and perchlorate represent alternative, more energetically favorable electron acceptors and can outcompete SRM for electron donors and thus limit SRM growth through this biocompetitive exclusion. Nitrate reduction can additionally result in the accumulation of nitrite, and perchlorate reduction can result in the accumulation of chlorite, both of which are potent SRM inhibitors (Carlson et al. 2014) and are therefore similar to a biocide. Effects of nitrate and perchlorate addition on the microbial ecology of sour reservoirs are elaborated upon below.

3 Nitrate Reduction and Perchlorate Reduction for the Control of Reservoir Souring

Nitrate is incorporated into waterflood regimes for many sour oil reservoirs to combat the activity of SRM. Like SRM, NRM can utilize various oil-derived electron donors, coupling their oxidation to the reduction of nitrate instead of sulfate. Hydrocarbons, organic acids, alcohols, and hydrogen can all be metabolized by different NRM. Furthermore, sulfide and other reduced sulfur compounds potentially present in the reservoir environment can also be coupled to the reduction of nitrate by chemolithotrophic NRM (e.g., nitrate-reducing sulfide-oxidizing microorganisms, NR-SOM) (Lahme et al. 2019). Hence nitrate reduction has the potential to prevent reservoir souring by competitive depletion of SRM substrates or by the direct oxidation of the harmful hydrogen sulfide (Fig. 1a). Thermodynamic modeling predicts that chemoorganotrophic NRM are favored over chemolithotrophic NR-SOM under reservoir conditions (Dolfing and Hubert 2017). Informed management of the sulfur cycle in nitrate-treated oil reservoirs depends on understanding and distinguishing between nitrate reduction pathways (Hubert et al. 2009). SRM activity can also be adversely affected by increased environmental redox potential due to the production of nitrate reduction intermediates (Nemati et al. 2001a). Nitrite is a main intermediate and has the added beneficial properties of inhibiting sulfate reduction directly as an alternative substrate of the dissimilatory sulfite reductase (Dsr) (Greene et al. 2003). Nitrate can also chemically scavenge sulfide that may be present (Sanders and Sturman 2005).

Perchlorate injection is an emerging approach to souring control that has many similarities with nitrate treatment. Perchlorate is utilized by perchlorate-reducing microorganisms (PRM) that can outcompete SRM for consumption of oil-derived electron donors (Coates et al. 1999; Carlström et al. 2013; Engelbrektson et al. 2018a, b). PRM can also oxidize sulfide, but primarily to elemental sulfur rather than to sulfur oxyanions (Gregoire et al. 2014; Mehta-Kolte et al. 2017). Elemental sulfur accumulation is a relevant consideration, given that it may be subsequently oxidized or reduced (Telang et al. 1999), and if it comes into contact with production facilities, it can exacerbate corrosion problems that are known to be associated with reservoir souring (Lahme and Hubert 2017; Lahme et al. 2019). While both nitrate and perchlorate can function as competitive inhibitors of sulfate reduction, perchlorate is a slightly more potent direct inhibitor of SRM (Carlson et al. 2014). Finally, reactive chlorine and oxygen species, like nitrite, are potent inhibitors of sulfate reduction (Carlson et al. 2014).

3.1 Chemoorganotrophic NRM, PRM, and Biocompetitive Exclusion

Depletion of SRM substrates by NRM as a souring control strategy is sometimes referred to as biocompetitive exclusion (Fig. 1a). This is based on nitrate reduction being more thermodynamically favorable than sulfate reduction when coupled to the

oxidation of a given substrate. Oil-field NRM stimulated by nitrate injection may, like SRM, be indigenous to the reservoir or introduced during water injection. Nitrate injection technology has gained broad attention from the petroleum industry only recently, and fewer oil-field NRM have been isolated and characterized, compared to SRM. Unlike oil-field SRM described so far, many characterized chemoorganotrophic NRM from oil fields can oxidize hydrocarbon electron donors (Ollivier and Cayol 2005). In order for biocompetitive exclusion to be successful, the NRM must oxidize the same substrates that the SRM would otherwise couple to the reduction of sulfate. Petroleum reservoirs represent complex microbial ecosystems and substrate utilization patterns for anaerobic respiration by oil-field SRM, and NRM will not necessarily be identical (Grigoryan and Voordouw 2008). For example, in one low-temperature oil field in western Canada, it has been observed that NRM oxidize aromatic hydrocarbons, whereas SRM predominantly use organic acids (Agrawal et al. 2012).

Similar to NRM, PRM outcompete SRM for electron donors due to the favorable thermodynamics of perchlorate reduction over sulfate reduction. Perchlorate is widespread in the environment and is present both as a contaminant and as a natural product owing to photooxidative processes in the upper atmosphere. PRM are therefore ubiquitous in the environment and present in both marine and freshwater systems (Coates et al. 1999; Carlström et al. 2013). In general, PRM are less abundant than NRM most likely due to the fact that nitrate is usually present at several orders of magnitude higher concentrations. However, while denitrification (nitrate reduction to N_2) provides five electrons per mole, perchlorate provides eight electrons per mole. Thus, from the standpoint of biocompetitive exclusion, fewer moles of perchlorate versus nitrate are required to consume the same amount of labile carbon sources and outcompete SRM if the nitrate reduction proceeds via denitrification. These points are well illustrated in flow-through column studies to assess the capacity of perchlorate and nitrate to inhibit sulfate reduction. While it takes longer for perchlorate to show an impact on sulfate reduction compared to nitrate, at equimolar concentrations, perchlorate is more effective than nitrate and inhibiting sulfate reduction (Coates 2014). Some oil-field NRM catalyze dissimilatory nitrate reduction to ammonia (DNRA) (Fig. 2a) (Hubert and Voordouw 2007), which also provides eight electrons per mole of nitrate. Further work is needed to address whether nitrate injection promotes DNRA or denitrification by NRM in different reservoir contexts.

PRM are also capable of using a variety of organic acids as well as crude oil hydrocarbons as electron donors and carbon sources to support growth. Of note, because PRM produce oxygen as an obligate intermediate of their anaerobic metabolism, PRM are able to activate hydrocarbons through aerobic pathways in anoxic environments (Coates et al. 1998; Carlström et al. 2013). This capacity for oxygenic hydrocarbon degradation may give PRM an additional advantage over SRM in oil reservoir environments.

If SRM causing reservoir souring can switch their metabolism to reduce the injected nitrate instead of sulfate (Seitz and Cypionka 1986; Dalsgaard and Bak 1994; Plugge et al. 2002), then sulfide production will stop, and electron donor

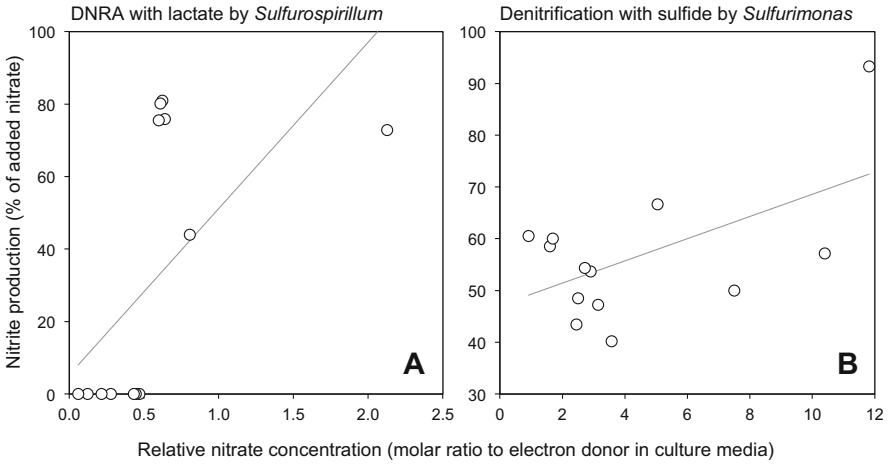

Fig. 2 Nitrite production as a function of nitrate concentration in pure culture tests using nitrate-reducing *Sulfurospirillum* sp. strain KW (**a**) and *Sulfurimonas* sp. strain CVO (**b**), both isolated from the Coleville oil field in western Canada. Chemoorganotrophic DNRA by *Sulfurospirillum* was governed by initial concentrations of nitrate relative to lactate (**a**). Chemolithotrophic denitrification by *Sulfurimonas* was governed by initial concentrations of nitrate relative to sulfide. Initial conditions in culture media (the relative nitrate concentration) are expressed as the ratio of nitrate to either lactate (**a**) or sulfide (**b**) on the x axes. High initial nitrate doses always corresponded to a greater proportion of the nitrate ending up as nitrite in final products as indicated by regression lines. Results in (**a**) and (**b**) are adapted from Hubert and Voordouw (2007) and Greene et al. (2003), respectively, which describe the individual pure culture experiments in more detail

depletion is not necessary. However, not all oil-field SRM can reduce nitrate (Greene et al. 2003). Many SRM also have mechanisms for detoxifying reactive nitrogen species (e.g., nitrite, nitric oxide). Similarly, some thermophilic SRM have been shown to reduce perchlorate (Liebensteiner et al. 2013, 2014), although the exact mechanisms for this remain obscure.

3.2 Sulfide Oxidation by Chemolithotrophic NRM and PRM

Several NRM isolated from oil-field environments couple nitrate reduction to the oxidation of sulfide and other reduced sulfur compounds (Fig. 1b) making them potentially beneficial souring-control agents. The product of sulfide oxidation may be sulfur compounds of an intermediate oxidation state (e.g., elemental sulfur) or sulfate. Intermediate sulfur compounds are corrosive (Nemati et al. 2001b; Hubert et al. 2005; Lahme and Hubert 2017; Lahme et al. 2019), and their accumulation may be prevented if microbes that reduce or oxidize elemental sulfur are present (Telang et al. 1999; Gevertz et al. 2000). Complete conversion of sulfide back to sulfate allows further sulfate reduction to occur if SRM electron donors remain available. In principle nitrate can deplete these electron donors via cycling of reduced and oxidized sulfur compounds between chemolithotrophic NRM and

sulfate or sulfur reducers (Fig. 1b). Hence, sulfide-oxidizing NRM can achieve the overall effect of biocompetitive exclusion in an indirect way that does not depend on oxidation of the exact SRM substrates that donate electrons to the souring reaction (Hubert et al. 2003). The ability of sulfide-oxidizing NRM to compete with chemoorganotrophic NRM for nitrate may determine the effectiveness of nitrate injection technology in instances where direct biocompetitive exclusion is precluded by resident SRM and NRM that oxidize different oil-derived substrates. Thermodynamic calculations suggest that chemoorganotrophs will outcompete chemolithotrophic NRM in souring control scenarios (Dolfing and Hubert 2017).

PRM are also innately capable of sulfide oxidation, but the products of the reaction are shifted toward elemental sulfur rather than sulfur oxyanions (Fig. 1) (Gregoire et al. 2014; Mehta-Kolte et al. 2017). This proceeds differently than sulfide oxidation by chemolithotrophic NRM in that PRM do not grow by sulfide oxidation; rather the intermediates and enzymes involved in perchlorate respiration are very reactive with sulfide (Mehta-Kolte et al. 2017). Organisms involved in the cycling of intermediate sulfur species are often observed co-enriched alongside PRM in marine sulfidogenic systems that have received perchlorate treatment to control souring (Coates 2014).

3.3 Nitrate Reduction Pathways and Production of Nitrite by Oil-Field NRM

The ability of NRM to control reservoir souring via depletion of SRM electron donors according to the scheme in Fig. 1a depends on the oxidative capacity of nitrate, which in turn depends on the NRM physiology and whether nitrate is completely or partially reduced. Nitrate reduction can proceed according to two reaction pathways: DNRA ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$), which transfers eight electrons, or denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), which transfers five electrons. Hence, oxidation (depletion) of electron donors would require less nitrate if full DNRA rather than full denitrification were stimulated. NRM capable of both processes have been isolated from the same oil field (Gevertz et al. 2000; Hubert and Voordouw 2007), and the factors governing which group may be stimulated during nitrate injection to reservoirs remain unclear. An experimental comparison using non-oil-field strains from a culture collection showed DNRA was more energetically favorable than denitrification (Strohm et al. 2007), whereas thermodynamic modeling predicts denitrification is more energetically favorable (Dolfing and Hubert 2017). It has been suggested that DNRA may be the dominant pathway in environments where nitrate concentrations are low (Burgin and Hamilton 2007).

However, nitrate reduction by oil-field NRM does not necessarily always proceed fully to the end products N_2 or NH_4^+. When nitrate is present in excess relative to NRM electron donors, nitrite may be the end product of nitrate reduction (Greene et al. 2003; Hubert and Voordouw 2007; Grigoryan et al. 2008). This is illustrated in NRM end-product plots for organic acid (lactate) oxidation by the DNRA-catalyzing *Sulfurospirillum* sp. strain KW and sulfide oxidation by the

denitrifying *Sulfurimonas* sp. strain CVO (Fig. 2). The results show that lower nitrate doses may be more likely to be converted to fully reduced end products, whereas higher doses promote nitrite accumulation. Instances could exist where adding less nitrate transfers more electrons (oxidizes more substrate) than higher nitrate doses that would be converted mainly to nitrite. This suggests that depletion of SRM electron donors could be more efficient using a relatively low dose of nitrate in some cases. More souring control with less nitrate seems counterintuitive, and strategies based on the trends shown in Fig. 2 require a thorough understanding of the chemistry and microbiology in a particular setting.

Nitrite production can be advantageous in oil-field settings since nitrite strongly inhibits SRM (Fig. 1). An important benefit of partial nitrate reduction scenarios (e.g., higher nitrate doses; Fig. 2) could be the production of significant amounts of nitrite, which appears to be a common outcome of nitrate application at high temperature (Fida et al. 2016). Some oil-field NRM produce only nitrite as an end product (e.g., sulfide-oxidizing *Arcobacter* sp. strain FWKOB (Gevertz et al. 2000)). Nitrite specifically blocks the dissimilatory sulfite reductase (Dsr) that catalyzes the conversion of sulfite to sulfide in all SRM (Greene et al. 2003) and is a moderately selective inhibitor of SRM (Carlson et al. 2015). As a metabolic inhibitor, nitrite is similar to a biocide and is more effective against SRM at higher concentrations. Nitrite inhibition of Dsr depends on SRM cell density; larger SRM populations require correspondingly higher nitrite doses to prevent growth and sulfide production (Haveman et al. 2004). Another important factor is whether or not the SRM being targeted encode and express a periplasmic nitrite reductase (Nrf). This feature, possessed by some but not all SRM, is effectively a detoxification strategy to alleviate nitrite inhibition of normal Dsr action (Greene et al. 2003); Nrf does not allow SRM growth via dissimilatory nitrite reduction (Pereira et al. 2000).

Introducing high doses of nitrate to sour reservoirs may thus achieve multiple souring control effects. Reduction of nitrate to nitrite transfers two electrons, which would achieve oxidation and depletion of SRM substrates in proportion to the amount of added nitrate. This outcome would be complemented by a corresponding level of inhibitory nitrite (Fig. 2). For oil fields where souring control depends mainly on inhibition of SRM by nitrite, it may be less crucial to understand the nature and extent of SRM electron donors in situ. However, recent work suggests that nitrite production in many oil systems may be primarily controlled by the availability of labile monoaromatic hydrocarbons such as toluene (Suri et al. 2017) that can be oxidized by betaproteobacterial *Azoarcus* and *Thauera* spp. detected in different oil fields (Hubert and Voordouw 2007; Li et al. 2014; Suri et al. 2017). Nitrite inhibition would also be effective in situations where culprit SRM are known to have a broad substrate range (perhaps including oil hydrocarbons) and/or in reservoirs with many degradable electron donors (making them harder to exhaust via nitrate-reducing biocompetitive exclusion). Torsvik and Sunde (2005) have suggested that oil has limitless electron donors (for SRM and NRM) and that souring control mechanisms must therefore be based on SRM inhibition. As such, information on resident microbial communities will be important for developing nitrite

inhibition strategies that depend on the amount of SRM biomass present, which can be estimated using quantitative assays (see Sect. 5). It may also be important to determine whether the SRM harbor nitrite reductase. Such information could be determined by pre-screening oil-field samples using molecular surveillance techniques prior to nitrate injection. If souring is caused by nitrite-resistant SRM, nitrite accumulation may be less successful, and a biocompetitive exclusion-based nitrate injection strategy for depleting bioavailable SRM electron donors should be considered. Recent work suggests that the extent of nitrite accumulation by NRM increases at high temperature (An et al. 2017) and salinity (Okpala et al. 2017). This could be due to inhibition of nitrite reductase enzymes at temperature and salinity extremes. The temperature sensitivity suggests that reinjection of hot production water could be used as a means of enhancing the efficacy of nitrate injection for souring control (An et al. 2017).

3.4 Perchlorate Reduction Pathways and Production of Oxygen and Reactive Chlorine Species by Perchlorate-Reducing Microorganisms

Perchlorate-reducing microorganisms are unique among respiratory anaerobes in that they can produce dioxygen under anaerobic conditions as an obligate intermediate in perchlorate reduction. All respiratory perchlorate-reducing microorganisms reduce perchlorate sequentially through the intermediates $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow O_2 \rightarrow H_2O$ (Youngblut et al. 2016b). Perchlorate (ClO_4^-) is first reduced through two sequential two-electron reductions to chlorite (ClO_2^-) by a perchlorate reductase (PcrA) protein (Youngblut et al. 2016a, b). In canonical PRM, perchlorate reductases form a monophyletic clade (Melnyk and Coates 2015), and biochemical studies suggest that although NarG nitrate reductases are capable of reducing perchlorate, the K_m for the specialized perchlorate reductase enzyme is several orders of magnitude lower (Youngblut et al. 2016a). Thus, PRM are capable of accessing much lower concentrations of perchlorate compared with nitrate-reducing microorganisms. The chlorite produced by PcrA is an exceptionally reactive and toxic intermediate and is rapidly dismutated to dioxygen and chloride ion by chlorite dismutase (Cld). Chlorite dismutation does not yield energy but produces dioxygen, a very thermodynamically favorable electron acceptor and oxidant. There are also a number of other specialized mechanisms, whereby perchlorate-reducing microorganisms detoxify reactive chlorine species such as hypochlorite (HOCl) that are formed through side reactions as minor products of perchlorate reduction. The presence of an active methionine sulfoxide reductase system in conjunction with a highly expressed methionine-rich peptide is essential to cope with these species in model PRM (Melnyk et al. 2015).

The dioxygen produced through perchlorate reduction is utilized by PRM using the same cytochrome oxidase enzymes that are utilized under aerobic conditions (Melnyk et al. 2013). This oxygen is also available to the enzymes involved in

aerobic hydrocarbon degradation (Carlström et al. 2015) and can potentially even be available to other organisms carrying out other aerobic metabolisms (Carlström et al. 2015; Clark et al. 2016) (Youngblut et al. 2016a; Barnum et al. 2018). The possibility of syntrophic perchlorate reduction has recently been demonstrated in pure culture (Clark et al. 2016), which suggests that PRM subpopulations could share the intermediates of perchlorate reduction. This could occur by exchange of either chlorite or dioxygen (Youngblut et al. 2016a). While this likely occurs at very low concentrations (low micromolar to nanomolar), both of these compounds are extremely potent inhibitors of SRM with IC_{50} values in that range (Carlson et al. 2015). Thus, as with NRM producing nitrite, PRM populations may exist that produce varying levels of chlorite and oxygen and thereby impact SRM to varying extents.

4 Alternative Oxyanion Treatment Strategies

Other oxyanion treatments for controlling reservoir souring have also been evaluated. While less is known about their application logistics and impact on complex microbial communities, considering the properties and impact of these other oxyanions on oil reservoir communities provides important points of comparison with nitrate and perchlorate and identifies scenarios where combined treatment strategies may work.

Nitrate and perchlorate can be effective inhibitors of microbial sulfate reduction in large part because of their capacity to serve as alternative electron acceptors and because NRM and PRM produce reactive nitrogen and chlorine species that are potent inhibitors of SRM (Fig. 1). However, both of these monovalent oxyanions can also function as competitive inhibitors of the ATP sulfurylase/sulfate adenylyltransferase (ATPS/Sat), and at sufficiently high concentrations, both compounds can function as direct, selective inhibitors of respiratory sulfate-reducing microorganisms (Carlson et al. 2014) (Fig. 3). Other oxyanions function as alternative substrates of the ATP sulfurylase and are activated to generate unstable adenosine 5′-phosphooxyanions with varying stabilities relative to adenosine 5′-phosphosulfate (APS) (Fig. 3).

Molybdate and tungstate, the group IV oxyanions, are particularly potent inhibitors of SRM because they form very unstable APS analogs (Peck 1959; Renosto et al. 1993; Hanna et al. 2004; Carlson et al. 2015). This is similarly true for selenate and presumably also true for tellurate (Renosto et al. 1993; Hanna et al. 2004; Carlson et al. 2015) (Peck 1959). Chromate and arsenate are also futile substrates of the sulfate adenylyltransferase, but this can be counteracted by enzymatic mechanisms for their detoxification in many SRM (Lovley and Phillips 1994; Michel et al. 2001; Li and Krumholz 2007). The net impact of these futile substrates is rapid depletion of cytoplasmic ATP and regeneration of the inhibitory oxyanion.

In contrast to the futile substrates, chromate and arsenate, monofluorophosphate forms a very stable APS analog (Hanna et al. 2004) that may also function as a

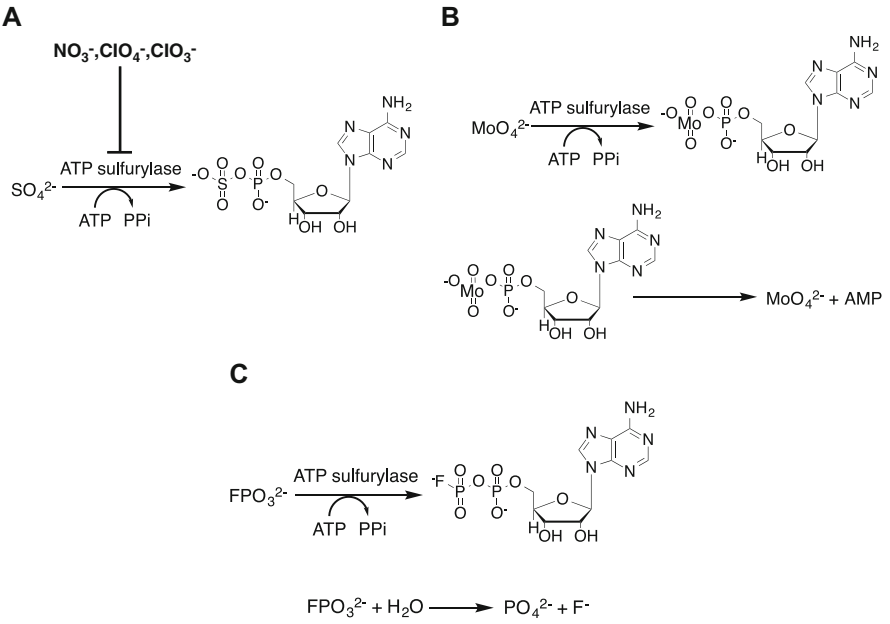

Fig. 3 Mechanisms of inhibition of sulfate adenylyltransferase/ATP sulfurylase (Sat) by inorganic oxyanions. (**a**) The monovalent oxyanions perchlorate, chlorate, and nitrate are competitive inhibitors of Sat, competing with sulfate for binding to the active site. (**b**) The divalent oxyanions molybdate, tungstate, selenate, tellurate, arsenate, and chromate are all alternative substrates of the Sat and are converted into unstable adenosine-5'-phosphosulfate analogs that rapidly decompose to release free oxyanion again. This drives a futile cycle with the net result of depleting cytoplasmic ATP in SRM. (**c**) Monofluorophosphate forms a uniquely stable adenosine-5'-phosphosulfate analog that may also function as a competitive inhibitor of the APS reductase. Cytoplasmic fluoride release in SRM also contributes to the mechanism of inhibition

competitive inhibitor of the APS reductase. Monofluorophosphate is also a selective inhibitor of SRM and functions in part by releasing toxic fluoride ion in the cytoplasm of SRM that are unable to distinguish between sulfate and monofluorophosphate (Carlson et al. 2015). The relatively low toxicity of monofluorophosphate to other organisms compared to the other inorganic oxyanions makes it an attractive option.

One consideration for the use of many of the divalent inorganic oxyanions is the potential for the formation of insoluble complexes with alkali earth metals such as Ca^{2+} and Mg^{2+} (Rowley and Stuckey 1956; Lide 2007). This could impact the ability of these inhibitors to move through reservoirs to reach target SRM populations but could also have the added effect of sequestering these inorganic oxyanions in mineral matrices that could then function to slowly release the inhibitor farther into the reservoirs. More research into the potential of other oxyanions for achieving souring control will shed light on these possibilities.

5 Monitoring SRM and NRM in Oil-Field Environments

The importance of SRM has led the petroleum industry to adopt different strategies for monitoring their occurrence in oil reservoirs and oil production facilities. Quantitative detection tools can provide useful information about SRM, e.g., before and after seawater injection and breakthrough which may allow SRM proliferation, or before and after biocide or nitrate treatments intended to curb SRM activity. Many souring control strategies depend on the inhibition and/or eradication of SRM; hence sensitive detection methods are useful for surveillance. Important considerations are the logistics of sample processing and the length of time until results are obtained and interpreted. Particular features relating to the microbial ecology of souring and nitrate addition, discussed above, may be important for correctly interpreting results when sulfide elimination is the objective. Advantages and disadvantages of different quantitative approaches are discussed below.

5.1 Cultivation-Based Monitoring

Traditionally the oil industry has employed most probable number (MPN) analyses, sometimes referred to as "bug bottles," for quantification of various microbial groups. Oil-field samples (e.g., produced waters, metal coupons from injection or production facilities, etc.) are inoculated into a medium containing sulfate and appropriate electron donors as dilution series and, following an incubation period, are scored based on set criteria (e.g., blackening caused by reaction of produced sulfide in iron-containing growth media). Detection of growth can require up to 4 weeks for highest positive dilutions, which in principle were inoculated with one to nine individual target cells (assuming tenfold dilution series and proportional biomass distribution during transfers, i.e., no clumping of cells). Increased sensitivity may be achieved by incubating with radioactive ^{35}S-labeled sulfate (Vester and Ingvorsen 1998). Similar methods, with appropriately selective growth media, can be employed for enumerating NRM, PRM, and other groups of oil-field microorganisms.

Detection of oil-field SRM based on the production of sulfide makes sense, since sulfide production is usually the problem in the first place. MPN testing generally does not return false-positive results; using appropriate SRM media, MPNs should reliably determine the minimum number of microbes capable of sulfate reduction that are present in a sample. A caveat to this would be samples with high concentrations of sulfide but low concentrations of SRM, underscoring the importance of incubating proper controls in parallel to SRM growth tubes. However, misinterpretation of positive results could occur, e.g., if SRM switch from fermentative metabolism to sulfate reduction, although they are not carrying out sulfate reduction in the reservoir, they may give a misleading result in the bug bottle tests. The necessary foreknowledge of media and incubation conditions that will successfully enrich reservoir microbes is a key drawback to MPN testing, given that most environmental microorganisms have not been successfully cultivated so far (Stewart 2012).

The so-called unculturable fraction is often suggested to be 90 to 99% of the microbial diversity in a given environment. This ratio presumably holds true for SRM in oil-field environments; hence there is real potential for false negatives using this approach. Typical oil-field MPN counts are 10^4 to 10^5 SRM ml^{-1} produced water (Birkeland 2005).

Dilution to extinction, inherent to the MPN approach, offers the opportunity to obtain pure cultures of culturable organisms associated with selected phenotypes in oil-field samples (e.g., Voordouw et al. 1991). Molecular methods cannot substitute live cultures for the experimental characterization of microbial physiology (e.g., determining the range of relevant phenotypes for a single organism). Pure cultures also allow straightforward whole-genome sequencing. Information from whole genomes offers valuable clues as to the metabolic potential of an organism, e.g., in an oil reservoir context, whether reactions, such as sulfate reduction, nitrate reduction, perchlorate reduction, corrosion-associated metabolism, or hydrocarbon biodegradation, could be catalyzed. In addition to obtaining genomes from pure cultures, community DNA sequencing of metagenomes to obtain whole genomes, or single-cell genomics following cell sorting, offers other ways to access this information from environmental (e.g., oil field) samples (Bowers et al. 2017) without culturing. Despite these developments in genomics, petroleum microbiology will continue to benefit from renewed cultivation efforts that employ innovative techniques and various culture media (Giovannoni and Stingl 2007; McGenity 2016). Expanding the number and diversity of cultured and well-characterized oil-field microorganisms is a good reason to maintain dilution-to-extinction (MPN) testing in the oil industry, but this requires that analyses performed by operators and service providers do not end with enumeration and continue all the way to individual strain isolation, characterization, and sequencing. Employing a wider array of selective growth media would be beneficial for this kind of initiative.

High-throughput cultivation can help petroleum microbiologists address many of the challenges related to accessing culturable diversity noted above. Coupled with high-throughput 16S rRNA gene amplicon sequencing, high-throughput MPN cultivation has been shown to be successful in this regard (Justice et al. 2017). Once microbial isolates and enrichments are obtained, high-throughput cultivation can allow researchers to rapidly identify appropriate biocide or oxyanion dosing concentrations and develop tailored souring control strategies specific to a given reservoir environment (Carlson et al. 2015, 2017). In addition, approaches for continuous cultivation, such as chemostats, are promising for obtaining stable microbial communities carrying out complex metabolisms such as sulfur and nitrogen cycling (Kraft et al. 2014).

5.2 Cultivation-Independent Monitoring

In recent years the oil industry has started incorporating molecular microbiology into its surveillance. At the same time, research labs have undertaken many molecular characterizations of oil-field production fluids, increasing our

understanding of reservoir microbial communities (Pham et al. 2009; Hubert et al. 2012; Lewin et al. 2014; Hu et al. 2016; Vigneron et al. 2017; Kim et al. 2018). While arguably more technically demanding than traditional MPN assays, molecular methods can provide quantitative results within hours to days rather than weeks. Differences on this timescale for managing oil production operations may translate into significant economic gain or loss. In this context it is essential to understand the reservoir microbial ecology in question, such that well-designed assays are implemented and their results interpreted correctly.

There are two main approaches used by molecular microbial ecologists for quantifying specific microbial groups of interest. Fluorescence in situ hybridization (FISH) of oligonucleotide probes to ribosomal RNA (rRNA) in viable cells after their fixation allows direct counting of active microorganisms by epifluorescence microscopy (Amann and Fuchs 2008). This approach has taxonomic specificity based on the probe sequence, whereas more general DNA-binding dyes (e.g., DAPI or SYBR Green) coupled with microscopy can provide a non-specific general cell count (though this can also count dead cells). These general stains are typically used in tandem with FISH so that organisms of interest can be considered relative to overall population estimates. A different approach is quantitative PCR (qPCR) where the progress of a PCR is optically monitored in real time using similar DNA-binding dyes (e.g., SYBR Green) such that the exponential growth in the fluorescence signal allows the initial number of target sequences in a sample to be extrapolated (Smith and Osborne 2008; McKew and Smith 2015; Shen and Voordouw 2015). Application of either technique for quantifying SRM or other microbial targets in oil-field samples requires careful selection of the genetic sequence(s) being targeted and the specificity of the oligonucleotide probe or primers being applied.

Non-quantitative molecular approaches that have been applied to oil-field samples include PCR-based amplicon libraries. In recent years, clone libraries, i.e., cloning amplicons into plasmid vectors for sequencing (e.g., Voordouw et al. 1996; Hubert et al. 2012) and denaturing gradient gel electrophoresis analysis of amplicons (Schwermer et al. 2008), have given way to next-generation sequencing approaches using different technology platforms, with the current state of the art for most researchers being the Illumina MiSeq platform (Dong et al. 2017; Vigneron et al. 2017). Amplicon libraries indicate the presence of some organisms but do not exclude the absence of others. Relative abundances of different taxonomic groups in amplicon libraries should be interpreted cautiously, because PCR primers tend to preferentially amplify certain taxa over others.

Maintaining up-to-date knowledge for oligonucleotide probes and PCR primers is an ongoing task given the rapid and regular discovery of new microbial diversity, both in oil fields and other environments. For example, one of the earliest FISH probes used for SRM detection in microbial ecology, SRB385, was designed to target some but not all known sulfate-reducing *Deltaproteobacteria* (Amann et al. 1990) at a time when 16S rRNA gene sequence databases and the molecular diversity of SRM (Vigneron et al. 2018) were more limited. This probe has gained attention from the oil industry for use in SRM surveillance. However, a quick and

easy analysis of SRB385 in the context of currently known 16S rRNA diversity using free online resources reveals perfect matches to thousands of known 16S rRNA sequences affiliated with non-sulfate-reducing groups, including inferred fermentative, syntrophic, and nitrate-reducing bacteria.

Probe and primer sequences, like growth media for the MPN approach, represent the selective component of molecular detection assays. Careful design and testing are crucial to proper probe and primer applications in oil-field settings. The SRB385 example illustrates the potential to overestimate SRM abundance using oligonucleotides with broader than intended specificity. On the other hand, specific probes or primer sets for SRM 16S rRNA targets may also lead to underestimates due to less than intended coverage and specificity for the target group. As noted above, SRM are phylogenetically diverse, belonging to at least five bacterial and two archaeal phyla (Stahl et al. 2009). In cases where specific SRM known to plague a particular oil field are to be monitored, a 16S rRNA gene sequence corresponding to that particular organism or clade may represent a good molecular marker for quantitative surveillance. Multiple 16S rRNA assays are required to confidently screen for all known SRM.

Alternatively, qPCR-based detection of metabolic genes of SRM such as the dissimilarity sulfite reductase (*dsr*AB) offers another strategy for specific detection of these organisms (Müller et al. 2015; Vigneron et al. 2018). While metabolic genes offer good targets for specific qPCR assays, FISH targeting metabolic genes has also been developed (Moraru et al. 2010; Barrero Canosa et al. 2017). Abundances of genes encoding enzymes catalyzing various steps in nitrate reduction pathways (Sect. 3.3) have also been assessed by qPCR in different environmental samples (Henry et al. 2006; Smith et al. 2007, 2017). Similarly, perchlorate reduction genes can also be detected in amplicon libraries (Bender et al. 2004) and in metagenomes (Barnum et al. 2018). Sulfide-oxidizing microorganisms that contain homologous *aps* and *dsr* genes to those in SRM and catalyze reverse reactions (Stahl et al. 2007) could potentially complicate interpretation of results from SRM metabolic gene assays from oil-field environments, especially in situations where, in response to SRM activity, nitrate injection stimulates sulfide-oxidizing NRM (Figs. 1b and 2b). Targeting *dsr* may hold more promise in this regard since it forms distinct clades in sulfate reducers and sulfide oxidizers (Loy et al. 2009), whereas *aps* from these two groups are phylogenetically interspersed (Stahl et al. 2007). Other caveats of *dsr* used as an oil-field marker are its occurrence among some fermentative and syntrophic *Desulfotomaculum* spp. that may have lost the ability to reduce sulfate (Plugge et al. 2002; Imachi et al. 2006) and the fact that some SRM are also capable of nitrate reduction (Seitz and Cypionka 1986; Dalsgaard and Bak 1994; Plugge et al. 2002). These organisms, if present, would still respond to *dsr*-based detection assays (or MPN assays using SRM growth medium) even when displaying these non-sulfate-reducing phenotypes in situ. Assays that monitor real-time generation of cDNA reverse transcribed from mRNA using a *dsr*-specific primer (Chin et al. 2008) could offer a work-around to avoid false-positive results from organisms not expressing their *dsr*; the success of this strategy would depend on sampling and RNA extraction protocols relative to the short half-life of microbial mRNA

(Frias-Lopez et al. 2008). Despite the alternatives that various metabolic gene targets offer for molecular surveillance, in oil-field microbiology, as in many other sub-disciplines of microbial ecology, 16S rRNA genes (for amplicon diversity surveys and qPCR) remain widely used biomarkers.

Another culture-independent strategy to monitor the activity of SRM in situ, rooted in biogeochemistry rather than in molecular biology, is to measure the isotopic shift in sulfate to detect sulfur cycling in the reservoir relative to injection waters (Hubert et al. 2009; Hubbard et al. 2014). While hydrogen sulfide production, sulfate consumption, or the presence of SRM may be difficult to detect, isotopic methods to measure the imprint of sulfur cycling via sulfate isotopic composition are extremely sensitive and can be used to detect early-stage souring. Adopting isotopic fractionation as a monitoring approach could give oil-field operators time to focus resources on problem reservoirs and implement the other approaches outlined above to identify actionable solutions and to monitor the efficacy of nitrate, perchlorate, or other treatments.

The best molecular monitoring will always result from a combination of various strategies including those discussed above. However, comprehensive approaches may not always be compatible with the important and "real-world" objectives of rapidly generating results that can inform field operators concerned with maintaining high oil production rates while minimizing harmful effects of souring. In some instances, thorough early-stage characterization that includes MPN enumerations and strain isolation may allow probe or primer selection for targeting important microorganisms known to be present, and responsive to perturbations, in a particular oil reservoir. In addition, microbial diversity assessments through amplicon library and/or metagenome sequencing are highly recommended as early as possible in the oil reservoir production life cycle, i.e., initial formation water samples obtained from production fluids during primary production, before injection of other fluids like seawater that can trigger reservoir souring by SRM. Similar microbiological analysis of the injection fluids is also recommended for a more thorough assessment of whether or not signatures in microbial diversity profiles are likely to represent organisms indigenous to the subsurface. Routine testing using carefully developed molecular strategies can be complemented by occasional H_2S-based MPN assays, stable isotope analyses, and additional SRM probe/primer sets to introduce degrees of quality assurance for the overall surveillance strategy.

6 Research Needs

Many oil companies have a good understanding of reservoir souring caused by SRM and have in recent years started introducing nitrate to alleviate souring problems at different production operations. In order for nitrate injection technology to be employed successfully in the years ahead, a more robust understanding of the underlying microbial ecology will be needed. The industry must move beyond a simple awareness that nitrate can work and gain the ability to distinguish NRM-based souring control mechanisms (Fig. 1) in different settings. This understanding will be instructive for troubleshooting instances when nitrate addition

stops working at given production operations. We may learn that reservoir microbial ecology relevant for oil production is largely specific for given oil fields. However, as more case studies are undertaken, common patterns will likely emerge. In this respect, case studies involving perchlorate are needed to assess its efficacy in the field, and these can be compared to nitrate injection case studies. Developing and applying the suite of surveillance tools discussed in Sect. 5, particularly the newer molecular methods and next-generation sequencing strategies, will continue to be an important and exciting feature of modern petroleum microbiology. The critical task of designing appropriate probes and primers for oil fields will parallel the practical goal of seeing these techniques adopted as routine aspects of microbiological monitoring by oil producers. Meeting these challenges will require a successful collaboration between microbiologists and oil producers seeking an improved understanding of microbial ecology in subsurface petroleum habitats.

Acknowledgments The authors wish to acknowledge the financial support from the Campus Alberta Innovates Program to CRJH and from the UC Berkeley Energy Biosciences Institute to HKC.

References

Agrawal A, Park HS, Nathoo S et al (2012) Toluene depletion in produced oil contributes to souring control in a field subjected to nitrate injection. Environ Sci Technol 46:1285–1292. https://doi.org/10.1021/es203748b

Alkindi A, Prince-Wright R, Moore WR et al (2013) Challenges for waterflooding in a Deepwater environment. SPE Prod Oper 23:404–410. https://doi.org/10.2118/118735-PA

Amann R, Fuchs BM (2008) Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques. Nat Rev Microbiol 6:339–348. https://doi.org/10.1038/nrmicro1888

Amann RI, Binder BJ, Olson RJ et al (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56:1919–1925

An BA, Shen Y, Voordouw G (2017) Control of sulfide production in high salinity bakken shale oil reservoirs by halophilic bacteria reducing nitrate to nitrite. Front Microbiol 8:1164. https://doi.org/10.3389/fmicb.2017.01164

Aüllo T, Ranchou-Peyruse A, Ollivier B, Magot M (2013) *Desulfotomaculum* spp. and related gram-positive sulfate-reducing bacteria in deep subsurface environments. Front Microbiol 4:362. https://doi.org/10.3389/fmicb.2013.00362

Barnum TP, Figueroa IA, Carlström CI et al (2018) Genome-resolved metagenomics identifies genetic mobility, metabolic interactions, and unexpected diversity in perchlorate-reducing communities. ISME J 115:E00E11–E01581. https://doi.org/10.1038/s41396-018-0081-5

Barrero Canosa J, Moraru C, Zeugner L et al (2017) Direct-geneFISH: a simplified protocol for the simultaneous detection and quantification of genes and rRNA in microorganisms. Environ Microbiol 19:70–82. https://doi.org/10.1111/1462-2920.13432

Barth T (1991) Organic acids and inorganic ions in waters from petroleum reservoirs, Norwegian continental shelf: a multivariate statistical analysis and comparison with American reservoir formation waters. Appl Geochem 6:1–15. https://doi.org/10.1016/0883-2927(91)90059-X

Bender KS, Rice MR, Fugate WH et al (2004) Metabolic primers for detection of (per)chlorate-reducing bacteria in the environment and phylogenetic analysis of cld gene sequences. Appl Environ Microbiol 70:5651–5658. https://doi.org/10.1128/AEM.70.9.5651-5658.2004

Birkeland N-K (2005) Sulfate-reducing bacteria and archaea. In: Petroleum microbiology. American Society of Microbiology, Washington DC, pp 35–54

Bowers RM, Kyrpides NC, Stepanauskas R et al (2017) Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol 35:725–731. https://doi.org/10.1038/nbt.3893

Bryant MP, Campbell LL, Reddy CA, Crabill MR (1977) Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H2-utilizing methanogenic bacteria. Appl Environm Microbiol 33:1162–1169

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89–96. https://doi.org/10.1890/1540-9295(2007)5[89:HWOTRO]2.0.CO;2

Carlson HK, Kuehl JV, Hazra AB et al (2014) Mechanisms of direct inhibition of the respiratory sulfate-reduction pathway by (per)chlorate and nitrate. ISME J 9:1295–1305. https://doi.org/10.1038/ismej.2014.216

Carlson HK, Stoeva MK, Justice NB et al (2015) Monofluorophosphate is a selective inhibitor of respiratory sulfate-reducing microorganisms. Environ Sci Technol 49:3727–3736. https://doi.org/10.1021/es505843z

Carlson HK, Mullan MR, Mosqueda LA et al (2017) High-throughput screening to identify potent and specific inhibitors of microbial sulfate reduction. Environ Sci Technol 51:7278–7285. https://doi.org/10.1021/acs.est.7b00686

Carlström CI, Wang O, Melnyk RA et al (2013) Physiological and genetic description of dissimilatory perchlorate reduction by the novel marine bacterium Arcobacter sp. strain CAB. mBio 4:e00217–e00213. https://doi.org/10.1128/mBio.00217-13

Carlström CI, Loutey D, Bauer S et al (2015) (Per)Chlorate-reducing bacteria can utilize aerobic and anaerobic pathways of aromatic degradation with (per)chlorate as an electron acceptor. mBio 6:e02287–e02214. https://doi.org/10.1128/mBio.02287-14

Chin K-J, Sharma ML, Russell LA et al (2008) Quantifying expression of a dissimilatory (bi)sulfite reductase gene in petroleum-contaminated marine harbor sediments. Microb Ecol 55:489–499. https://doi.org/10.1007/s00248-007-9294-2

Clark IC, Youngblut M, Jacobsen G et al (2016) Genetic dissection of chlorate respiration in *Pseudomonas stutzeri* PDA reveals syntrophic (per)chlorate reduction. Environ Microbiol 18:3342–3354. https://doi.org/10.1111/1462-2920.13068

Coates JD (2014) Inhibition of microbial sulfate reduction in a flow-through column system by (per) chlorate treatment. 1–11. https://doi.org/10.3389/fmicb.2014.00315/abstract

Coates JD, Bruce RA, Haddock JD (1998) Anoxic bioremediation of hydrocarbons. Nature 396:730–730. https://doi.org/10.1038/25470

Coates JD, Michaelidou U, Bruce RA et al (1999) Ubiquity and diversity of dissimilatory (per) chlorate-reducing bacteria. Appl Environ Microbiol 65:5234–5241

Dalsgaard T, Bak F (1994) Nitrate reduction in a sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, isolated from rice paddy soil: sulfide inhibition, kinetics, and regulation. Appl Environ Microbiol 60:291–297

Dolfing J, Hubert CRJ (2017) Using thermodynamics to predict the outcomes of nitrate-based oil reservoir souring control interventions. Front Microbiol 8:2575. https://doi.org/10.3389/fmicb.2017.02575

Dong X, Kleiner M, Sharp CE, et al (2017) Fast and simple analysis of MiSeq amplicon sequencing data with MetaAmp. bioRxiv:131631. https://doi.org/10.1101/131631

Eckford RE, Fedorak PM (2004) Chapter 11 Using nitrate to control microbially-produced hydrogen sulfide in oil field waters. In: Petroleum biotechnology – developments and perspectives. Elsevier, Oxford, pp 307–340

Engelbrektson AL, Cheng Y, Hubbard CG, Jin YT, Arora B, Tom LM, Hu P, Grauel AL, Conrad ME, Andersen GL, Ajo-Franklin JB, Coates JD (2018a) Attenuating Sulfidogenesis in a soured continuous flow column system with perchlorate treatment. Front Microbiol 9:1575. https://doi.org/10.3389/fmicb.2018.01575

Engelbrektson A, Briseno V, Liu Y, Figueroa I, Yee M, Shao GL, Carlson H, Coates JD (2018b) Mitigating Sulfidogenesis with simultaneous perchlorate and nitrate treatments. Front Microbiol 9:2305. https://doi.org/10.3389/fmicb.2018.02305

Fida TT, Chen C, Okpala G, Voordouw G (2016) Implications of limited thermophilicity of nitrite reduction for control of sulfide production in oil reservoirs. Appl Environ Microbiol 82:4190–4199. https://doi.org/10.1128/AEM.00599-16

Frias-Lopez J, Shi Y, Tyson GW et al (2008) Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci U S A 105:3805–3810. https://doi.org/10.1073/pnas.0708897105

Gevertz D, Telang AJ, Voordouw G, Jenneman GE (2000) Isolation and characterization of strains CVO and FWKO B, two novel nitrate-reducing, sulfide-oxidizing bacteria isolated from oil field brine. Appl Environ Microbiol 66:2491–2501. https://doi.org/10.1128/AEM.66.6.2491-2501.2000

Gieg LM, Jack, TR, Foght JM (2011) Appl Microbiol Biotechnol 92:263. https://doi.org/10.1007/s00253-011-3542-6

Giovannoni S, Stingl U (2007) The importance of culturing bacterioplankton in the "omics" age. Nat Rev Micro 5:820–826. https://doi.org/10.1038/nrmicro1752

Gittel A, Sørensen KB, Skovhus TL et al (2009) Prokaryotic community structure and sulfate reducer activity in water from high-temperature oil reservoirs with and without nitrate treatment. Appl Environ Microbiol 75:7086–7096. https://doi.org/10.1128/AEM.01123-09

Greene EA, Hubert C, Nemati M et al (2003) Nitrite reductase activity of sulphate-reducing bacteria prevents their inhibition by nitrate-reducing, sulphide-oxidizing bacteria. Environ Microbiol 5:607–617. https://doi.org/10.1046/j.1462-2920.2003.00446.x

Greene EA, Brunelle V, Jenneman GE, Voordouw G (2006) Synergistic inhibition of microbial sulfide production by combinations of the metabolic inhibitor nitrite and biocides. Appl Environ Microbiol 72:7897–7901. https://doi.org/10.1128/AEM.01526-06

Gregoire P, Engelbrektson A, Hubbard CG et al (2014) Control of sulfidogenesis through bio-oxidation of H2S coupled to (per)chlorate reduction. Environ Microbiol Rep 6:558–564

Grigoryan A, Voordouw G (2008) Microbiology to help solve our energy needs. Ann N Y Acad Sci 1125:345–352. https://doi.org/10.1196/annals.1419.004

Grigoryan AA, Cornish SL, Buziak B et al (2008) Competitive oxidation of volatile fatty acids by sulfate- and nitrate-reducing bacteria from an oil field in Argentina. Appl Environ Microbiol 74:4324–4335. https://doi.org/10.1128/AEM.00419-08

Hanna E, Ng KF, MacRae IJ et al (2004) Kinetic and stability properties of *Penicillium chrysogenum* ATP sulfurylase missing the C-terminal regulatory domain. J Biol Chem 279:4415–4424. https://doi.org/10.1074/jbc.M311317200

Harms G, Zengler K, Rabus R et al (1999) Anaerobic oxidation of o-xylene, m-xylene, and homologous alkylbenzenes by new types of sulfate-reducing bacteria. Appl Environ Microbiol 65:999–1004

Haveman SA, Greene EA, Stilwell CP et al (2004) Physiological and gene expression analysis of inhibition of Desulfovibrio vulgaris Hildenborough by nitrite. J Bacteriol 186:7944–7950. https://doi.org/10.1128/JB.186.23.7944-7950.2004

Head IM, Jones DM, Larter SR (2003) Biological activity in the deep subsurface and the origin of heavy oil. Nature 426:344–352. https://doi.org/10.1038/nature02134

Henry S, Bru D, Stres B et al (2006) Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Appl Environ Microbiol 72:5181–5189. https://doi.org/10.1128/AEM.00231-06

Hu P, Tom L, Singh A et al (2016) Genome-resolved metagenomic analysis reveals roles for candidate phyla and other microbial community members in biogeochemical transformations in oil reservoirs. mBio 7:e01669–e01615. https://doi.org/10.1128/mBio.01669-15

Hubbard CG, Cheng Y, Engelbrekston A et al (2014) Isotopic insights into microbial sulfur cycling in oil reservoirs. Front Microbiol 5:480. https://doi.org/10.3389/fmicb.2014.00480

Hubert C, Voordouw G (2007) Oil field souring control by nitrate-reducing Sulfurospirillum spp. that outcompete sulfate-reducing bacteria for organic electron donors. Appl Environ Microbiol 73:2644–2652. https://doi.org/10.1128/AEM.02332-06

Hubert C, Nemati M, Jenneman G, Voordouw G (2003) Containment of biogenic sulfide production in continuous up-flow packed-bed bioreactors with nitrate or nitrite. Biotechnol Prog 19:338–345. https://doi.org/10.1021/bp020128f

Hubert C, Nemati M, Jenneman G, Voordouw G (2005) Corrosion risk associated with microbial souring control using nitrate or nitrite. Appl Microbiol Biotechnol 68:272–282. https://doi.org/10.1007/s00253-005-1897-2

Hubert C, Voordouw G, Mayer B (2009) Elucidating microbial processes in nitrate- and sulfate-reducing systems using sulfur and oxygen isotope ratios: the example of oil reservoir souring control. Geochim Cosmochim Acta 73:3864–3879. https://doi.org/10.1016/j.gca.2009.03.025

Hubert CRJ, Oldenburg TBP, Fustic M, Gray ND, Larter SR, Penn K, Rowan AK, Seshadri R, Sherry A, Swainsbury R, Voordouw G, Voordouw J, Head IM (2012) Massive dominance of *Epsilonproteobacteria* in formation waters from a Canadian oil sands reservoir containing severely biodegraded oil. Environ Microbiol 14:387–404

Imachi H, Sekiguchi Y, Kamagata Y et al (2006) Non-sulfate-reducing, syntrophic bacteria affiliated with Desulfotomaculum cluster I are widely distributed in methanogenic environments. Appl Environ Microbiol 72:2080–2091. https://doi.org/10.1128/AEM.72.3.2080-2091.2006

Jensen AB, Webb C (1995) Treatment of H2S-containing gases: a review of microbiological alternatives. Enzym Microb Technol 17:2–10. https://doi.org/10.1016/0141-0229(94)00080-B

Johnson RJ, Folwell BD, Wirekoh A et al (2017) Reservoir souring – latest developments for application and mitigation. J Biotechnol 256:57–67. https://doi.org/10.1016/j.jbiotec.2017.04.003

Jones DM, Head IM, Gray ND et al (2008) Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. Nature 451:176–180. https://doi.org/10.1038/nature06484

Justice NB, Sczesnak A, Hazen TC, Arkin AP (2017) Environmental selection, dispersal, and organism interactions shape community assembly in high-throughput enrichment culturing. Appl Environ Microbiol 83:e01253–e01217. https://doi.org/10.1128/AEM.01253-17

Kim DD, O'Farrell C, Toth CRA, Montoya O, Gieg LM, Kwon TH, Yoon S (2018) Microbial community analyses of produced waters from high-temperature oil reservoirs reveal unexpected similarity between geographically distant oil reservoirs. Microb Biotechnol 11:788–796

Kraft B, Tegetmeyer HE, Sharma R et al (2014) Nitrogen cycling. The environmental controls that govern the end product of bacterial nitrate respiration. Science 345:676–679. https://doi.org/10.1126/science.1254070

Lahme S, Hubert C (2017) Corrosion risks associated with (bio)chemical processes in sour systems due to nitrate injection or oxygen ingress. In: Enning DE, Skovhus TL, Lee J (eds) Microbiologically influenced corrosion in the upstream oil and gas industry. Taylor and Francis, Boca Raton, pp 87–109. ISBN: 978-149872660-3; 978-149872656-6

Lahme S, Enning DE, Callbeck CM, Menendez Vega D, Curtis TP, Head IM, Hubert CRJ (2019) Metabolites of an oil field sulfide-oxidizing nitrate-reducing *Sulfurimonas* sp. cause severe corrosion. Appl Environ Microbiol 85(3):01891–01818. https://doi.org/10.1128/AEM.01891-18

Lewin A, Johansen J, Wentzel A et al (2014) The microbial communities in two apparently physically separated deep subsurface oil reservoirs show extensive DNA sequence similarities. Environ Microbiol 16:545–558. https://doi.org/10.1111/1462-2920.12181

Li X, Krumholz LR (2007) Regulation of arsenate resistance in *Desulfovibrio desulfuricans* G20 by an arsRBCC operon and an arsC gene. J Bacteriol 189:3705–3711. https://doi.org/10.1128/JB.01913-06

Li G, Gao P, Wu Y et al (2014) Microbial abundance and community composition influence production performance in a low-temperature petroleum reservoir. Environ Sci Technol 48:5336–5344. https://doi.org/10.1021/es500239w

Lide DD (2007) CRC handbook of chemistry and physics, 88th edn. CRC Press, Boca Raton, pp 1–59

Liebensteiner MG, Pinkse MWH, Schaap PJ et al (2013) Archaeal (per)chlorate reduction at high temperature: an interplay of biotic and abiotic reactions. Science 340:85–87. https://doi.org/10.1126/science.1233957

Liebensteiner MG, Tsesmetzis N, Stams AJM, Lomans BP (2014) Microbial redox processes in deep subsurface environments and the potential application of (per)chlorate in oil reservoirs. Front Microbiol 5:428. https://doi.org/10.3389/fmicb.2014.00428

Lovley DR, Phillips EJP (1994) Reduction of chromate by Desulfovibrio vulgaris and its c3 cytochrome. Appl Environ Microbiol 60:726–728

Loy A, Duller S, Baranyi C et al (2009) Reverse dissimilatory sulfite reductase as phylogenetic marker for a subgroup of sulfur-oxidizing prokaryotes. Environ Microbiol 11:289–299. https://doi.org/10.1111/j.1462-2920.2008.01760.x

Magot M (2005) Indigenous microbial communities in oil fields. In: Petroleum microbiology. American Society of Microbiology, Washington DC, pp 21–34

McGenity TJ (2016) Introduction to the isolation and cultivation of microbes involved in the hydrocarbon cycle. In: Hydrocarbon and lipid microbiology protocols, 2nd edn. Springer Berlin Heidelberg, Berlin/Heidelberg, pp 1–25

McKew BA, Smith CJ (2015) Real-time PCR approaches for analysis of hydrocarbon-degrading bacterial communities. In: Hydrocarbon and lipid microbiology protocols. Springer Berlin Heidelberg, Berlin/Heidelberg, pp 45–64

Mehta-Kolte MG, Loutey D, Wang O et al (2017) Mechanism of H2S oxidation by the dissimilatory perchlorate-reducing microorganism Azospira suillum PS. mBio 8:e02023–e02016. https://doi.org/10.1128/mBio.02023-16

Melnyk RA, Coates JD (2015) The perchlorate reduction genomic island: mechanisms and pathways of evolution by horizontal gene transfer. BMC Genomics 16:616. https://doi.org/10.1186/s12864-015-2011-5

Melnyk RA, Clark IC, Liao A, Coates JD (2013) Transposon and deletion mutagenesis of genes involved in perchlorate reduction in Azospira suillum PS. mBio 5:e00769–e00713. https://doi.org/10.1128/mBio.00769-13

Melnyk RA, Youngblut MD, Clark IC et al (2015) Novel mechanism for scavenging of hypochlorite involving a periplasmic methionine-rich peptide and methionine sulfoxide reductase. mBio 6:e00233–15–e00233–18. https://doi.org/10.1128/mBio.00233-15

Michel C, Brugna M, Aubert C et al (2001) Enzymatic reduction of chromate: comparative studies using sulfate-reducing bacteria: key role of polyheme cytochromes c and hydrogenases. Appl Microbiol Biotechnol 55:95–100. https://doi.org/10.1007/s002530000467

Moraru C, Lam P, Fuchs BM et al (2010) GeneFISH – an in situ technique for linking gene presence and cell identity in environmental microorganisms. Environ Microbiol 12:3057–3073. https://doi.org/10.1111/j.1462-2920.2010.02281.x

Müller AL, Kjeldsen KU, Rattei T et al (2015) Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. ISME J 9:1152–1165. https://doi.org/10.1038/ismej.2014.208

Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Micro 6:441. https://doi.org/10.1038/nrmicro1892

Nemati M, Jenneman GE, Voordouw G (2001a) Mechanistic study of microbial control of hydrogen sulfide production in oil reservoirs. Biotechnol Bioeng 74:424–434. https://doi.org/10.1002/bit.1133

Nemati M, Jenneman GE, Voordouw G (2001b) Impact of nitrate-mediated microbial control of souring in oil reservoirs on the extent of corrosion. Biotechnol Prog 17:852–859. https://doi.org/10.1021/bp010084v

Okpala GN, Chen C, Fida T, Voordouw G (2017) Effect of thermophilic nitrate reduction on sulfide production in high temperature oil reservoir samples. Front Microbiol 8:1573. https://doi.org/10.3389/fmicb.2017.01573

Ollivier B, Cayol J-L (2005) Fermentative, iron-reducing, and nitrate-reducing microorganisms. In: Petroleum microbiology. American Society of Microbiology, Washington DC, pp 71–88

Ollivier B, Cayol J-L, Fauque G (2009) Sulphate-reducing bacteria from oil field environments and deep-sea hydrothermal vents. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria. Cambridge University Press, Cambridge, pp 305–328

Oremland RS, Capone DG (1988) Use of "specific" inhibitors in biogeochemistry and microbial ecology. In: Advances in microbial ecology. Springer US, Boston, pp 285–383

Pannekens M, Kroll L, Müller H, Tall Mbow F, Meckenstock RU (2019) Oil reservoirs, an exceptional habitat for microorganisms. New Biotechnol 49:1–9

Peck HD (1959) The ATP-Dependent Reduction of Sulfate with Hydrogen in Extracts of Desulfovibrio desulfuricans. Proc Natl Acad Sci U S A 45:701–708. https://doi.org/10.1073/pnas.45.5.701

Pereira IA, LeGall J, Xavier AV, Teixeira M (2000) Characterization of a heme c nitrite reductase from a non-ammonifying microorganism, *Desulfovibrio vulgaris* Hildenborough. Biochim Biophys Acta 1481:119–130

Pham VD, Hnatow LL, Zhang S et al (2009) Characterizing microbial diversity in production water from an Alaskan mesothermic petroleum reservoir with two independent molecular methods. Environ Microbiol 11:176–187. https://doi.org/10.1111/j.1462-2920.2008.01751.x

Plugge CM, Balk M, Stams AJM (2002) *Desulfotomaculum thermobenzoicum* subsp. thermosyntrophicum subsp. nov., a thermophilic, syntrophic, propionate-oxidizing, spore-forming bacterium. IJSEB 52:391–399. https://doi.org/10.1099/00207713-52-2-391

Postgate JR (1952) Competitive and noncompetitive inhibitors of bacterial sulphate reduction. J Gen Microbiol 6:128–142. https://doi.org/10.1099/00221287-6-1-2-128

Renosto F, Patel HC, Martin RL et al (1993) ATP sulfurylase from higher plants: kinetic and structural characterization of the chloroplast and cytosol enzymes from spinach leaf. Arch Biochem Biophys 307:272–285. https://doi.org/10.1006/abbi.1993.1590

Rowley HH, Stuckey JE (1956) Preparation and properties of calcium monofluorophosphate dihydrate. J Am Chem Soc 78:4262–4263. https://doi.org/10.1021/ja01598a022

Sanders PF, Sturman PJ (2005) Biofouling in the oil industry. In: Petroleum microbiology. American Society of Microbiology, Washington DC, pp 171–198

Schwermer CU, Lavik G, Abed RMM et al (2008) Impact of nitrate on the structure and function of bacterial biofilm communities in pipelines used for injection of seawater into oil fields. Appl Environ Microbiol 74:2841–2851. https://doi.org/10.1128/AEM.02027-07

Seitz H-JR, Cypionka H (1986) Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrite. Arch Microbiol 146:63–67. https://doi.org/10.1007/BF00690160

Shen Y, Voordouw G (2015) Primers for dsr genes and most probable number method for detection of sulfate-reducing bacteria in oil reservoirs. In: Shen Y, Voordouw G (eds) Hydrocarbon and lipid microbiology protocols. Springer Berlin Heidelberg, Berlin/Heidelberg, pp 35–43

Smith CJ, Nedwell DB, Dong LF, Osborn AM (2007) Diversity and abundance of nitrate reductase genes (narG and napA), nitrite reductase genes (nirS and nrfA), and their transcripts in estuarine sediments. Appl Environ Microbiol 73:3612–3622. https://doi.org/10.1128/AEM.02894-06

Smith CJ, Osborne AM (2008) Advantages and limitations of quantitative PCR(Q-PCR)-based approaches in microbial ecology. FEMS Microbiol Ecol 67:6–20

Smith CJ, McKew BA, Coggan A, Whitby C (2017) Primers: functional genes for nitrogen-cycling. In: McGenity TJ et al (eds) Hydrocarbon and lipid microbiology protocols, Springer protocols handbooks, pp 207–241. https://doi.org/10.1007/8623_2015_184

Stahl DA, Loy A, Wagner M (2007) Molecular strategies for studies of natural populations of sulphate-reducing microorganisms. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria. Cambridge University Press, Cambridge, pp 39–116

Stahl DA, Loy A, Wagner M (2009) Molecular strategies for studies of natural populations of sulphate-reducing microorganisms. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria. Cambridge University Press, Cambridge, pp 39–116

Stewart EJ (2012) Growing unculturable bacteria. J Bacteriol 194:4151–4160. https://doi.org/10.1128/JB.00345-12

Strohm TO, Griffin B, Zumft WG, Schink B (2007) Growth yields in bacterial denitrification and nitrate ammonification. Appl Environ Microbiol 73:1420–1424. https://doi.org/10.1128/AEM.02508-06

Suri N, Voordouw J, Voordouw G (2017) The effectiveness of nitrate-mediated control of the oil field sulfur cycle depends on the toluene content of the oil. Front Microbio 8:956. https://doi.org/10.3389/fmicb.2017.00956

Telang AJ, Ebert S, Foght JM et al (1998) Effects of two diamine biocides on the microbial community from an oil field. Can J Microbiol 44:1060–1065. https://doi.org/10.1139/cjm-44-11-1060

Telang AJ, Jenneman GE, Voordouw G (1999) Sulfur cycling in mixed cultures of sulfide-oxidizing and sulfate- or sulfur-reducing oil field bacteria. Can J Microbiol 45:905–913. https://doi.org/10.1139/cjm-45-11-905

Thrasher DR, Vance I (2005) Reservoir souring: mechanisms and prevention. In: Petroleum microbiology. American Society of Microbiology, Washington DC, pp 123–142

Torsvik T, Sunde E (2005) Microbial control of hydrogen sulfide production in oil reservoirs. In: Petroleum microbiology. American Society of Microbiology, Washington DC, pp 201–213

Vester F, Ingvorsen K (1998) Improved most-probable-number method to detect sulfate-reducing bacteria with natural media and a radiotracer. Appl Environ Microbiol 64:1700–1707

Vigneron A, Alsop EB, Lomans BP et al (2017) Succession in the petroleum reservoir microbiome through an oil field production lifecycle. ISME J 11:2141–2154. https://doi.org/10.1038/ismej.2017.78

Vigneron A, Cruaud P, Alsop E, de Rezende JR, Head IM (2018) Beyond the tip of the iceberg; A new view of the diversity of sulfite- and sulfate-reducing microorganisms. ISME J 12:2096–2099

Voordouw G, Armstrong SM, Reimer MF et al (1996) Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. Appl Environ Microbiol 62:1623–1629

Voordouw G, Voordouw JK, Karkhoff-Schweizer RR, Fedorak PM, Westlake DWS (1991) Reverse Sample Genome Probing, a New Technique for Identification of Bacteria in Environmental Samples by DNA Hybridization, and Its Application to the Identification of Sulfate-Reducing Bacteria in Oil Field Samples. Appl Environ Microbiol 57(11):3070–3078

Widdel F, Musat F, Knittel K, Galushko A (2009) Anaerobic degradation of hydrocarbons with sulphate as electron acceptor. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria. Cambridge University Press, Cambridge, pp 265–304

Youngblut MD, Tsai C-L, Clark IC et al (2016a) Perchlorate reductase is distinguished by active site aromatic gate residues. J Biol Chem 291:9190–9202. https://doi.org/10.1074/jbc.M116.714618

Youngblut MD, Wang O, Barnum TP, Coates JD (2016b) (Per)chlorate in biology on earth and beyond. Annu Rev Microbiol 70:435–457. https://doi.org/10.1146/annurev-micro-102215-095406

Zengler K, Richnow HH, Rosselló-Mora R et al (1999) Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 401:266–269. https://doi.org/10.1038/45777

13 Microbial Communities in Oil Sands Tailings: Their Implications in Biogeochemical Processes and Tailings Management

Tariq Siddique, Sebastian Stasik, Mohd Faidz Mohamad Shahimin, and Katrin Wendt-Potthoff

Contents

T. Siddique (✉)
Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada
e-mail: tariq.siddiqu@ualberta.ca

S. Stasik
Department of Lake Research, UFZ – Helmholtz Centre for Environmental Research, Magdeburg, Germany

Faculty of Medicine Carl Gustav Carus, Technische Universität, Dresden, Germany
e-mail: Sebastian.Stasik@uniklinikum-dresden.de

M. F. Mohamad Shahimin
Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada

Department of Chemical Engineering Technology, Universiti Malaysia Perlis, Padang Besar, Perlis, Malaysia
e-mail: mohdfaid@ualberta.ca; faidz.shahimin@gmail.com

K. Wendt-Potthoff
Department of Lake Research, UFZ – Helmholtz Centre for Environmental Research, Magdeburg, Germany
e-mail: katrin.wendt-potthoff@ufz.de

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_10

Abstract

Bitumen extraction from surface-mined oil sands ores at a gigantic scale produces enormous volumes of fluid fine tailings (FFT) as a waste that are deposited in oil sands tailings ponds (OSTP). Increasing footprint of OSTP and related environmental consequences have drawn public scrutiny and warrant effective management of FFT. OSTP harbor diverse microbial communities that drive many biogeochemical processes in OSTP. In this chapter, we describe the microbial pathways of methanogenesis, and sulfur, nitrogen, and iron transformations in tailings that mitigate toxicity of organic constituents through biodegradation, accelerate consolidation of FFT, and regulate greenhouse gas emissions from OSTP. These microbial processes can also affect FFT reclamation under end-pit-lake (wet) scenario. Understanding microbial and geochemical composition of tailings will help design better strategies for utilizing tailings products for upland (dry) reclamation as well.

1 Introduction

In northeastern Alberta Canada, oil sands, a heavily biodegraded crude oil in the form of bitumen, represent one of the world's largest proven petroleum reserves (Larter and Head 2014) and contribute more than 90% of the total crude oil production in Canada (National Energy Board; https://www.neb-one.gc.ca/nrg/sttstc/crdlndptrlmprdct/index-eng.html). Bitumen extraction, upgrading, and export represent a large component of Canada's economy and domestic energy security. On the other hand, there are environmental consequences of oil sands development (described in detail by Foght et al. 2017). Current oil sands production (two–three million barrels day^{-1}) is expected to reach four million barrels day^{-1} by 2024 (http://www.energy.alberta.ca/OurBusiness/oilsands.asp). Shallow oil sands deposits ($\leq$50–75 m below surface; mbs) in the oil sands region are suitable for surface mining that produces a waste known as fluid tailings, whereas in situ steam assisted gravity drainage (SAGD) is used to recover bitumen from deeper deposits by injecting hot steam in the reservoir to liquefy bitumen (Foght et al. 2017). The scale of operations is gigantic representing the largest mining operation in the world. The total land area disturbed by active mining and tailings containment was estimated to be 895 km^2 in 2013 (Burkus et al. 2014), and the volume of impounded fluid fine tailings (FFT; see Box 1) is currently >1 billion m^3 (http://osip.alberta.ca/map/), while new and future mining operations (such as Fort Hills and Lewis projects) are in development.

Box 1 Acronyms

BTEX	Benzene, toluene, ethylbenzene, and isomers of xylene
EPL	End pit lakes; a tailings wet reclamation scenario currently being field-tested
FFT	Fluid fine tailings; having low solids content (e.g., $\leq$8 wt%)
GHG	Greenhouse gases primarily methane (CH_4) and carbon dioxide (CO_2)
MFT	Mature fine tailings; having solids content $\geq$30 wt%
MLSB	Mildred Lake Settling Basin; the largest, one of the oldest, and the most-studied OSTP
NAs	Naphthenic acids; alkyl-substituted acyclic and cycloaliphatic carboxylic acids
OSPW	Oil sands process-affected water, including the aqueous surface layer of OSTP
OSTP	Oil sands tailings ponds; man-made impoundments containing solid and fluid by-products of oil sands surface mining and bitumen extraction
PAH	Polycyclic aromatic hydrocarbons
SRB	Sulfate-reducing bacteria
TT	Thickened tailings; tailings that have been mixed with organic polymer to flocculate the solids and recover water
WIP	West In-Pit; Syncrude Canada's tailings pond (now Base Mine Lake; BML)

The oil sands industry is engaged on two fronts: (1) reclamation of mine disturbed land and (2) management of legacy FFT retained in oil sands tailings ponds (OSTP) and the FFT being generated (~1 million m^3 day^{-1}) during the bitumen extraction process (Siddique et al. 2014a). The environmental issues associated with OSTP are emission of biogenic greenhouse gases (GHG) primarily methane (CH_4) and carbon dioxide (CO_2); presence of residual hydrocarbons, naphthenic acids (NAs; complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids), polycyclic aromatic hydrocarbons (PAH), and trace metals; and slow settling of clays in FFT accompanied by poor recovery of porewater from the deposited FFT (Foght et al. 2017). OSTP harbor diverse microbial communities (Penner and Foght 2010; Siddique et al. 2012; An et al. 2013a; Saidi-Mehrabad et al. 2013; Mohamad Shahimin and Siddique 2017a, b) actively involved in various interlinked biogeochemical processes affecting all of these environmental aspects. Anaerobic microbial metabolism of residual hydrocarbons is the source of GHG emissions from OSTP (Siddique et al. 2006, 2007, 2011; Mohamad Shahimin and Siddique 2017a, b) and can be gauged and modeled to predict GHG emissions from OSTP (Siddique et al. 2008; Burkus et al. 2014; Kong et al. unpublished data). Other elemental cycling,

such as sulfur (S) and iron (Fe) (described in more detail in Sects. 3.2 and 3.4), can affect methanogenesis, the process causing GHG emissions. Microbial metabolism and associated biogenic gas production have also been linked with accelerated consolidation of MFT and recovery of porewater both in situ and in the laboratory (Holowenko et al. 2000; Guo 2009; Siddique et al. 2014a, b). OSTP microbial communities function under different redox conditions prevailed at different depths in OSTP and may degrade other toxic constituents such as some NAs and PAH (Clothier and Gieg 2016; Rochman et al. 2017).

The importance of microbial activities in OSTP was overlooked until recently when new regulations in Alberta, Canada, were put in place requiring oil sands industry to reduce FFT footprint, which leads to exploration of both "wet" and "dry" reclamation scenarios. Construction of end pit lakes (EPL) by burying mature fine tailings (MFT) under fresh and oil sands process affected water (OSPW) is considered a feasible wet reclamation option (Burkus et al. 2014), and microbial activities in underlying MFT will have profound effect on the viability of EPLs. Production of thickened tailings (TT) by de-watering FFT through chemical or physical manipulations for dry reclamation under upland landscape scenario may generate microbially aided acid rock drainage (ARD) depending on the contents of sulfide minerals in TT (Kuznetsov et al. 2015, 2016). Therefore, it is important to understand the role of microorganisms in tailings geochemical processes. Environmental consequences of oil sands development provide unique scientific opportunities to examine the role of microbes in this massive engineered environment. Currently microbes manifest as both beneficial and deleterious agents in the management and reclamation of oil sands mining wastes. To understand their roles in various aspects of OSTP and reclamation practices, it is necessary to understand the processes that generate oil sands mining wastes and influence tailings management strategies.

2 Brief Overview of Bitumen Extraction from Oil Sands Ore, Generation of Oil Sands Tailings, and Characteristics of Oil Sands Tailings Ponds

Foght et al. (2017) give the detailed information on the composition of oil sands ores and the bitumen extraction process. Oil sands ores comprise ~10 wt% bitumen, ~5% water, and ~85% quartz sand, silt, and clays (Chalaturnyk et al. 2002). Ores are crushed and mixed with hot (historically 70–80 °C but now ~50 °C) or warm (35–40 °C) water at pH ~8.5 to recover bitumen. The ore:water slurry is agitated to produce a bitumen-rich froth at the surface. A light hydrocarbon diluent is typically added during froth treatment to enhance bitumen recovery from the mineral matrix. Most of the diluent is recovered from the froth treatment tailings before they are deposited, along with other tailings streams, in enormous mined-out pits to create OSTP. The sand settles rapidly, typically forming surface or subsurface beaches of "coarse tailings" at the pond margins, whereas fresh FFT (comprising ~ 8 wt% fines, ~5% unextracted bitumen, <1% unrecovered diluent and ~85% process water) form a colloidal suspension in OSTP. Initially, FFT de-waters (recovery of interstitial

water with consolidation of mineral particles by gravity) relatively quickly reaching ~15 wt% solids within a few weeks and ~20% solids after 3–9 months. Afterwards, porewater expression becomes slower and produces MFT when solids content reaches ≥30 wt% during 5–10 years after deposition. Further self-weight natural consolidation would take >125 years (if external interventions are not employed) to produce "trafficable" material (Eckert et al. 1996) with substantial shear strength for reclamation. Throughout this settling period, OSPW is expressed from pore spaces and accumulates at the pond surface to be reclaimed for re-use in bitumen extraction process.

Hydrocarbon sheens and suspended or floating bitumen globules are common on the surface of OSTP. The thickness of OSPW layer at the pond surface may vary from 2 to 10 m. The uppermost layer of water is oxic due to wind and wave action, but the bulk water rapidly becomes anoxic with depth (Ramos-Padrón et al. 2011; Saidi-Mehrabadi et al. 2013; Stasik et al. 2014). Below the OSPW zone, a diffuse "mudline," an interface between the water layer and solids-enriched FFT layer, exists with a rapidly increasing density gradient downward from <0.1 wt% to >10% solids content. Below the mudline, the FFT gradually increase in solids content to become MFT, and the total depth below the mudline may be ≥40 m. Besides increasing solids content, other physical and chemical properties also change with depth. Temperature typically increases with depth, for example, 12 °C at 6 mbs to 22 °C at 30 mbs in Syncrude's (Syncrude Canada Ltd.) Mildred Lake Settling Basin (MLSB) (Penner and Foght 2010) and 7 °C at the surface to 19 °C at 18.3 mbs in Suncor's (Suncor Energy) Pond 6 (Ramos-Padrón et al. 2011), due to the deposition of warm fresh tailings and the retention of heat possibly generated from microbial metabolism in this dense and insulating material. Chemical gradients may also exist, with diluent hydrocarbons and soluble electron acceptors like sulfate typically decreasing with depth (age) compared to surface layers where fresh input replenishes these components (Guo 2009; Penner and Foght 2010, Stasik et al. 2014).

FFT and MFTs in the tailings ponds are mostly anoxic, attributable to the loss of oxygen (O_2) due to microbial metabolism, higher temperature, and mass transfer limitation with OSTP depth. Therefore, only strict and/or facultative anaerobic microorganisms can thrive in FFT and MFT. Since OSTP contain unrecovered hydrocarbons, anaerobic microorganisms in FFT and MFT utilize the hydrocarbons as carbon source under fermentative, nitrate (NO_3^-)-reducing, iron (Fe^{III})-reducing, sulfate (SO_4^{2-})-reducing, or methanogenic conditions. Composition of microbial communities in OSTP from different oil sands operators may vary due to the striking differences in the age of OSTP and the methods they employed for bitumen extraction and tailings consolidation. Syncrude, Suncor, and CNRL (Canadian Natural Resources Ltd.) use naphtha as the diluent, a petroleum distillate comprising aliphatic hydrocarbons of ~C_5–C_{16} and monoaromatics (BTEX: benzene, toluene, ethylbenzene and xylene isomers); however, Albian (Albian Sands Energy Inc.) and Imperial (Imperial Oil Ltd.) use a light paraffinic diluent comprising mainly C_5–C_6 *n*- and *iso*-alkanes (Burkus et al. 2014). The tailings management methods also differ among operators to enhance tailings consolidation: Albian mixes both trisodium

citrate and organic polymer flocculants with tailings before deposition (Li 2010), whereas CNRL injects CO_2 into tailings (www.cnrl.com/). These factors contribute towards differences in physicochemical properties of tailings generated by different operators, which may directly influence the microbial processes and community compositions.

3 Biogeochemical Processes and Microorganisms in Oil Sands Tailings Under Anaerobic Conditions

3.1 Methanogenesis and Methane Emissions from Oil Sands Tailings

In early 1990s, MLSB, which is one of Alberta's largest and oldest tailings ponds, started emitting CH_4 (estimated at ~40 million L d^{-1}) to the atmosphere after more than a decade of operation (Holowenko et al. 2000). Methanogenesis is a complex anaerobic process involving bacteria and archaeal methanogens in syntrophic relation (Fig. 1), which is sustained by hydrocarbons from unrecovered extraction diluent and bitumen entrained in tailings deposited in OSTP (Siddique et al. 2006, 2007, 2011, 2012; Mohamad Shahimin and Siddique 2017a, b).

Though bitumen is a complex mixture of high molecular weight hydrocarbons recalcitrant to biodegradation, some components such as longer chain *n*-alkanes

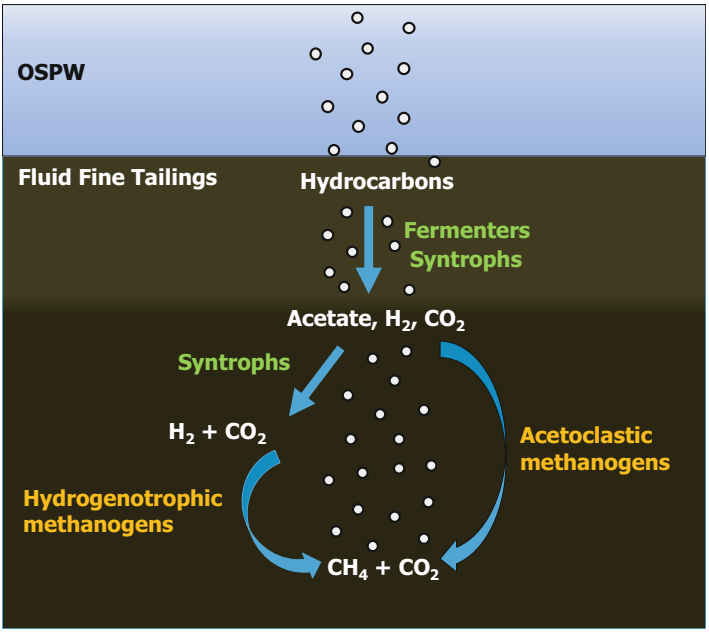

Fig. 1 Methanogenic hydrocarbon biodegradation pathway. (Modified from Siddique et al. 2012)

(C_{14}–C_{18}) could be a source of biogenic CH_4 in OSTP (Fig. 2c) (Siddique et al. 2011). The primary source of methanogenesis in OSTP is the extraction diluent that contains light hydrocarbons, labile substrates for microbial metabolism for CH_4 production. A year-long earlier study, where MFT collected from MLSB and spiked with naphtha or its individual fractions separately, produced CH_4. Short-chain *n*-alkanes (C_6–C_{10}) and some monoaromatics (toluene, *o*-xylene and *m*-, *p*-xylenes in BTEX mixture) were metabolized to CH_4 (Fig. 2a, b) (Siddique et al. 2006, 2007) revealing the source of methanogenesis in OSTP. Other naphtha fractions, such as branched- (*iso*-) and cyclic- (cyclo-) alkanes, remained recalcitrant to biodegradation during the year-long incubation (Siddique et al. 2007).

However, subsequent studies using MFT from Syncrude, CNRL, and Albian OSTP reveal that indigenous microbes in MFT during longer incubations can completely biodegrade major *iso*-alkanes (2-methylpentane, 3-methylhexane, 2-methylheptane, 4-methylheptane, 2-methyloctane, 3-methyloctane, 4-methyloctane, and 2-methylnonane) present in naphtha and paraffinic solvent (Abu Laban et al. 2015; Siddique et al. 2015; Tan et al. 2015; Mohamad Shahimin and Siddique 2017a, b; Siddique et al. unpublished data). These collective findings suggest that methanogenic biodegradation of hydrocarbons in MFT occurs in a sequence based on structure, where *n*-alkanes and monoaromatics are biodegraded first followed by *iso*-alkanes and then some cycloalkanes (Siddique et al. 2007; Mohamad Shahimin and Siddique 2017a, b). In addition to this sequential biodegradation, microbes also exhibit preference within a group of similar hydrocarbons. Microorganisms in Syncrude and CNRL MFT preferentially biodegrade longer-chain *n*-alkanes (biodegradation proceeds from nC_{10} to nC_5), whereas microbes in Albian MFT prefer nC_5 and nC_6 to nC_8 and nC_{10} *n*-alkanes for biodegradation (Siddique et al. 2006; Mohamad Shahimin et al. 2016). This microbial biodegradation behavior could be related to the microbial acclimation to the composition of hydrocarbons present in those OSTP: Syncrude and CNRL use naphtha as diluent that contains *n*-alkanes in the range of C_6–C_{10} versus Albian OSTP that receives tailings with residual paraffinic solvent containing shorter *n*-alkanes (C_5–C_6). Though not a part of the scope of this section (methanogenesis), it would be worth mentioning here that molecular structures do influence the biodegradability of organic compounds even under aerobic condition. For example, various studies investigated biodegradation of NAs (toxic constituents of OSPW) under aerobic conditions and revealed that vulnerability to biodegradation depended on alkyl side chain branching, intramolecular hydrogen bonding (*trans*- versus *cis*-isomers), and cyclization (Headley et al. 2002; Han et al. 2008; Smith et al. 2008; Johnson et al. 2011).

In OSTP, methanogenesis is performed by indigenous microorganisms. Initial enumerations of microorganisms in tailings from various tailings ponds revealed diverse anaerobic prokaryotes including denitrifiers, iron-reducers, sulfate-reducers, and methanogens suggesting the presence of potential hydrocarbon-degraders in oil sands tailings (Fedorak et al. 2002). Foght et al. (2017) comprehensively reviewed the microbiota in OSTP from different oil sands operators, showing high anaerobic archaeal and bacterial diversity. However, because anaerobic microorganisms only biodegrade a restricted range of hydrocarbons (Widdel et al. 2010), different microbial players were expected to dominate the microbial community during

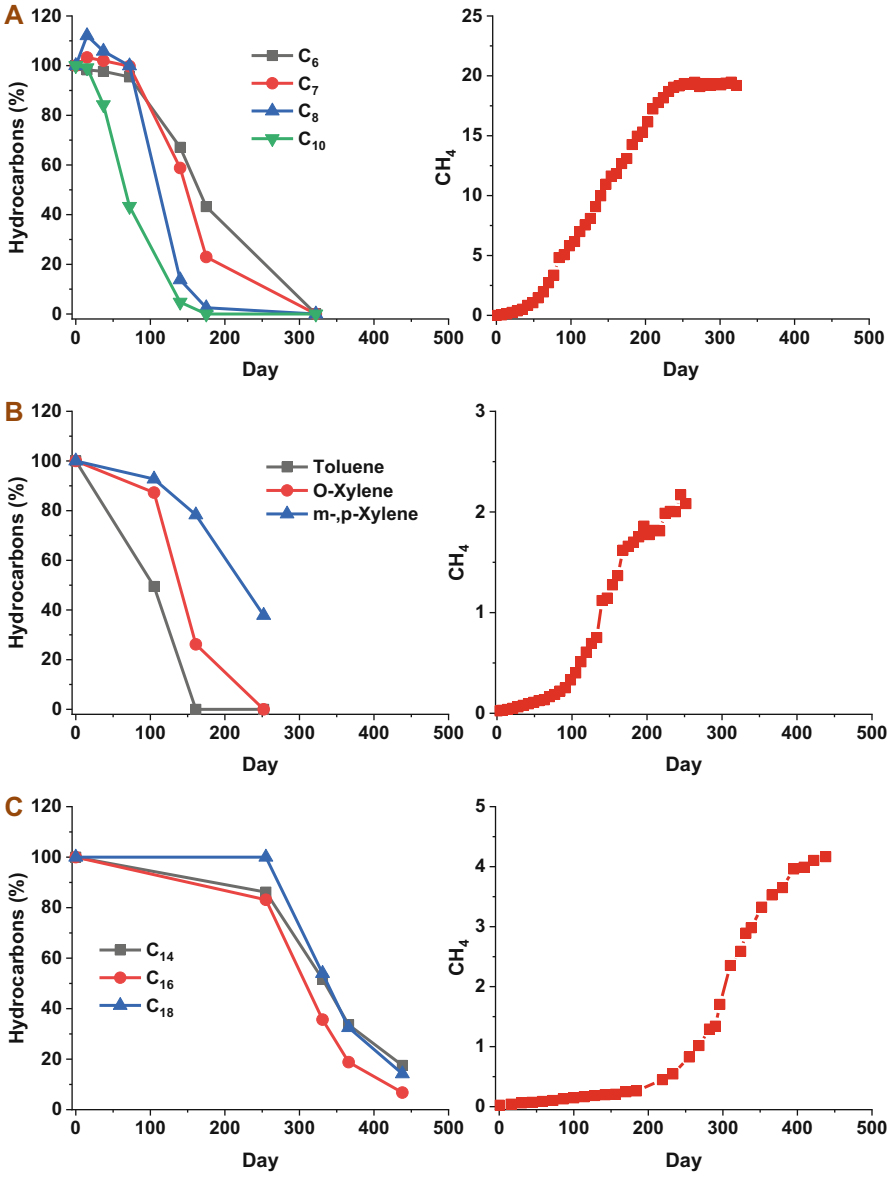

Fig. 2 Microbial metabolism of hydrocarbons into CH_4 in MFT collected from Mildred Lake Settling Basin (MLSB). (**a**) Biodegradation of short-chain *n*-alkanes (C_6–C_{10}) and (**b**) monoaromatics as components of naphtha entrained in tailings. (**c**) Biodegradation of longer-chain *n*-alkanes (C_{14}–C_{18}) as components of *n*-alkane fraction of unrecovered bitumen in tailings. This figure has been constructed retrieving the data from the work reported by Siddique et al. (2006, 2007, 2011). The data show the sequential biodegradation where short-chain *n*-alkanes (C_6–C_{10}) and some monoaromatics were biodegraded by 300 days (panels **a** and **b**). Biodegradation of longer *n*-alkanes (C_{14}–C_{18}) occurred between 300 and 450 days (panel **c**). Major *iso*-alkanes (C_7–C_8) were metabolized after 450 days in Syncrude MFT. (Data not shown; Siddique et al. unpublished data)

biodegradation of different hydrocarbons. Therefore, in this chapter, the focus is on the key microbial players that were enriched during the biodegradation of various groups of hydrocarbons or diluents indicating their involvement in the biodegradation process.

Dominant taxa of microorganisms enriched during hydrocarbon biodegradation in MFT from different OSTP are given in Table 1. In Syncrude MFT, members of *Firmicutes* (*Peptococcaceae*) and *Proteobacteria* (*Syntrophus/Smithella*) became dominant among bacteria during biodegradation of short-chain *n*-alkanes (C_6–C_{10}), monoaromatics (toluene and xylenes), and whole naphtha, and labile fractions of naphtha such as *n*-alkanes and monoaromatics (Siddique et al. 2012). *Peptococcaceae* also completely dominated the bacterial community during shorter *n*-alkane (C_5–C_6) and *iso*-alkane (C_5–C_8) biodegradation (Abu Laban et al. 2015; Siddique et al. 2015; Tan et al. 2015). *Syntrophus/Smithella* outnumbered all other bacterial taxa while biodegrading longer-chain *n*-alkanes (C_{14}–C_{18}) in Syncrude MFT (Siddique et al. 2011). In CNRL MFT, the bacterial community was completely dominated by *Peptococcaceae* in MFT amended with shorter-chain *n*- and *iso*-alkanes (C_5–C_6) (Mohamad Shahimin et al. 2016; Mohamad Shahimin and Siddique, unpublished data) as well as in MFT amended with paraffinic solvent that was entirely composed of *n*- and *iso*-alkanes in the range of C_5–C_6 (Mohamad Shahimin and Siddique 2017a). However, for the biodegradation of higher carbon chain hydrocarbons, the bacterial communities were dominated by *Syntrophaceae* for C_5–C_8 *iso*-alkane biodegradation (Mohamad Shahimin and Siddique, unpublished data), or co-dominated by *Anaerolineaceae, Syntrophaceae*, and *Desulfobacteraceae* for the biodegradation of C_6–C_{10} *n*-alkanes (Mohamad Shahimin et al. 2016) and naphtha (Mohamad Shahimin and Siddique 2017b). A visible trend of the enrichment of *Peptococcaceae* in MFT for shorter C_5–C_6 alkanes and *Syntrophaceae* with other partners such as *Desulfobacteraceae* and *Anaerolineaceae* in MFT for longer chain alkanes (C_6–C_{18}) highlights the restricted ability of microbes to metabolize certain hydrocarbons. Similar results in terms of microbial community composition were observed in Albian MFT where exclusive enrichment of *Peptococcaceae* or in some cases with *Anaerolineaceae* was observed during the biodegradation of all the hydrocarbon mixtures (Table 1), because Albian OSTP receives paraffinic solvent (C_5–C_6 alkanes) and the bacterial community in Albian MFT is acclimatized to metabolize a narrow range of hydrocarbons. On the other hand, CNRL and Syncrude use naphtha as diluent, a higher range hydrocarbons, accommodating a more diverse bacterial community (*Syntrophaceae, Desulfobacteraceae, Peptococcaceae* and *Anaerolineaceae*). All the bacterial taxa enriched during biodegradation of hydrocarbons in all MFT have been implicated as primary-hydrocarbon degraders in many cultures grown on various hydrocarbons under various reducing conditions (Zengler et al. 1999; Grundmann et al. 2008; Gray et al. 2011; Cheng et al. 2013; Abu Laban et al. 2015; Fowler et al. 2014; Liang et al. 2015; Siddique et al. 2011, 2012, 2015; Tan et al. 2014a, b, 2015; Mohamad Shahimin et al. 2016).

The archaeal communities in all MFT were dominated exclusively by methanogens. Acetoclastic methanogens (*Methanosaetaceae*) dominated in all MFT amended with short-chain *n*-alkanes (C_5–C_{10}) suggesting acetoclastic

methanogenesis as the primary methanogenic pathway during biodegradation. Other MFT amended with complex hydrocarbons (diluents), BTEX, long-chain *n*-alkanes (C_{14}–C_{18}), and some *iso*-alkanes exhibited co-domination of acetoclastic and hydrogenotrophic (members of *Methanomicrobiales*) methanogens (Table 1). Since biodegradation of hydrocarbons under methanogenic conditions involves complex syntrophic relationship between bacteria and methanogens (Fig. 1), both acetoclastic and hydrogenotrophic methanogenesis are important to keep the biodegradation process thermodynamically feasible. However, one methanogenesis pathway may be more dominant than the other depending on concentrations of acetate, hydrogen, and formate produced during the initial biodegradation process of the hydrocarbons. It has not been tested if methylotrophic methanogenesis, the production of methane from methylated substrates, can be relevant in oil sands tailings in situ. However, methylotrophic methanogens such as *Methanomethylovorans* have been detected in

Table 1 Key microbial players enriched during biodegradation of different hydrocarbon groups in different MFTs

MFT	Amendment*	Bacteria	Archaea
Syncrude	*n*-Alkanes (C_6–C_{10})[a,1]	*Clostridia* (*Peptococcaceae*) & *Syntrophus/Smithella*	*Methanosaetaceae*
	BTEX[a,1]	*Clostridia* (*Peptococcaceae*) & *Syntrophus/Smithella*	*Methanomicrobiales* & *Methanosaetaceae*
	Naphtha (Syncrude) [a,1]	*Clostridia* (*Peptococcaceae*) & *Syntrophus/Smithella*	*Methanosaetaceae* & *Methanomicrobiales*
	n-Alkanes (C_{14}–C_{18})[a,2]	*Syntrophus/Smithella*	*Methanosarcinales* & *Methanomicrobiales*
	iso-Alkanes (C_5–C_8)[3,4]	*Peptococcaceae*	*Candidatus* Methanoregula & *Methanosaetaceae*
	n-Alkanes (C_5–C_6)[4]	*Peptococcaceae*	*Candidatus* Methanoregula & *Methanosaetaceae*
CNRL	*n*-Alkanes (C_5–C_6)[5]	*Peptococcaceae*	*Methanosaetaceae*
	n-Alkanes (C_6–C_{10})[5]	(i) *Anaerolineaceae, Syntrophaceae* & *Desulfobacteraceae.* (ii) *Peptococcaceae, Syntrophaceae* & *Desulfobacteraceae.*	(i) *Methanosaetaceae* & *Candidatus* Methanoregula. (ii) *Candidatus* Methanoregula & *Methanosaetaceae*
	Naphtha (CNRL)[6]	(i) *Anaerolineaceae* & *Syntrophaceae.* (ii) *Peptococcaceae* & *Anaerolineaceae*	(i) *Methanosaetaceae* & *Candidatus* Methanoregula. (ii) *Candidatus* Methanoregula & *Methanosaetaceae*
	Paraffinic solvent (Albian)[7]	*Peptococcaceae*	*Methanosaetaceae* & *Candidatus* Methanoregula
	iso-Alkanes (C_5–C_6)[8]	*Peptococcaceae*	*Methanosaetaceae*
	iso-Alkanes (C_5–C_8)[8]	*Syntrophaceae*	*Methanosaetaceae*

(*continued*)

Table 1 (continued)

MFT	Amendment*	Bacteria	Archaea
Albian	*n*-Alkanes (C_5–C_6)[5]	*Peptococcaceae*	*Methanosaetaceae*
	n-Alkanes (C_6–C_{10})[5]	*Peptococcaceae* & *Anaerolineaceae*	*Methanosaetaceae*
	Naphtha (CNRL)[6]	*Peptococcaceae* & *Anaerolineaceae*	*Methanosaetaceae* & *Candidatus* Methanoregula
	Paraffinic solvent (Albian)[7]	(i) *Anaerolineaceae* (ii) Peptococcaceae	(i) *Methanosaetaceae* & *Methanosarcinaceae.* (ii) *Candidatus* Methanoregula
	iso-Alkanes (C_5–C_6)[4]	*Peptococcaceae*	*Candidatus* Methanoregula & *Methanosaetaceae*
	iso-Alkanes (C_5–C_6)[8]	*Peptococcaceae*	*Methanosaetaceae*

*All microbial 16S rRNA sequences were analyzed using 454 pyrosequencing except the hydrocarbon groups labelled "a" which were analyzed using gene clone libraries. (i) Composition of microbial community during early stage of biodegradation. (ii) Composition of microbial community after significant components of hydrocarbon mixtures have been depleted

[1]Siddique et al. 2012
[2]Siddique et al. 2011
[3]Abu Laban et al. 2015; Tan et al. 2015
[4]Siddique et al. 2015
[5]Mohamad Shahimin et al. 2016
[6]Mohamad Shahimin and Siddique 2017b
[7]Mohamad Shahimin and Siddique 2017a
[8]Mohamad Shahimin and Siddique unpublished data

MFT from Syncrude's WIP and MLSB, and their potential activity, studied as trimethylamine conversion, even exceeded acetogenic methanogenesis in some tailings enrichments (Penner and Foght 2010; Bordenave et al. 2010).

Studies were also conducted to investigate how indigenous microbes in OSTP biodegrade hydrocarbons under methanogenic conditions. Several anaerobic hydrocarbon activation mechanisms have been postulated in recent years namely: (1) carboxylation, (2) hydroxylation, and (3) fumarate addition (Heider and Schuhle 2013). Methanogenic hydrocarbon activation via carboxylation and hydroxylation pathways is still debatable due to lack of enough evidences regarding their metabolites and functional genes. The evidences for fumarate addition pathway have been reported in many studies involving anaerobic hydrocarbon biodegradation under various reducing conditions including methanogenic conditions (Rabus et al. 2001; Callaghan et al. 2006; Foght 2008; Boll and Heider 2010; Mbadinga et al. 2011; Zedelius et al. 2011; Callaghan 2013; von Netzer et al. 2013). To elucidate the major activation pathway in OSTP, initial screening of functional genes via metagenomics has revealed diverse postulated functional genes involved in activation of monoaromatic compounds via carboxylation and fumarate addition in tailings retrieved from Suncor's Tailings Pond 6 at various depths (An et al. 2013b). Metagenomic analysis of a methanogenic MFT enrichment culture grown on short-chain alkanes

(C_6–C_{10}) detected homologs of putative succinate synthase genes (e.g., *assA*, *bssA*, and *nmsA*) that further indicated fumarate addition as the main pathway of alkane activation under methanogenic conditions. Homologs of putative benzene carboxylase and ethylbenzene dehydrogenase genes, which are involved in activation via carboxylation and hydroxylation, respectively, were also detected, but the evidence was not conclusive (Tan et al. 2013). Recent studies on methanogenic *iso*- and cycloalkane biodegradation in MFT (Abu Laban et al. 2015; Tan et al. 2015) identified metabolites that corroborated the existence of functional genes involved in fumarate addition. The draft genome sequences of *Smithella* (Tan et al. 2014a) and *Peptococcaceae* (Tan et al. 2014b) enriched during methanogenic biodegradation of monoaromatics or alkanes in MFT also detected putative fumarate-addition pathway genes in these bacteria. Findings based on metagenomics and functional gene analysis suggested the fumarate addition pathway as the main activation pathway of hydrocarbon biodegradation under methanogenic conditions in OSTP.

The current findings and on-going experimentation examining biodegradation of various hydrocarbons in different MFT are important in improving our understanding of anaerobic hydrocarbon biodegradation processes in tailings ponds. Integration of such information into the existing mathematical model (Siddique et al. 2008) will result in better prediction of the potential GHG emissions from different OSTP. This first approximate kinetic model was developed based on a range of fugitive biodegradable hydrocarbons (Siddique et al. 2006, 2007) using stoichiometric conversion of hydrocarbons to CH_4, biodegradation rate of biodegradable hydrocarbons in MFT, and microbial carbon conversion efficiency (Siddique et al. 2008). However, later long-term studies revealed the biodegradation of additional components of fugitive naphtha and aliphatic diluent such as *iso*-alkanes (Abu Laban et al. 2015; Siddique et al. 2015; Mohamad Shahimin and Siddique 2017a, b). Therefore, a wider range of biodegradable hydrocarbons, additional microbial growth and death rates, and growth-limiting nutrients such as nitrogen (N) are being incorporated to further improve the mathematical model for effective GHG prediction (Kong et al. unpublished data). Other terminal electron acceptors such as sulfate (SO_4^{2-}), nitrate (NO_3^-) and Fe^{III} (discussed in Sects. 3.2, 3.3, and 3.4, respectively) if present or introduced in OSTP can also impact CH_4 emissions from OSTP. As an example, the activity of sulfate-reducing bacteria (SRB) may have prevented the release of approximately 5.37 million L of CH_4 day^{-1} from the former West In-Pit (WIP) (Stasik and Wendt-Potthoff 2016).

3.2 Sulfur Cycling

In some OSTP (particularly Suncor ponds), S cycling is promoted by the presence of SO_4^{2-}, which is added to OSTP in the form of gypsum ($CaSO_4{\cdot}2H_2O$) (approximately 1 kg per m^3) in order to increase dewatering and densification of FFT. As a consequence, SO_4^{2-} concentrations are typically elevated (2–6 mM) in the oxic surface waters (1–3 m) and decrease rapidly in anoxic tailings due to the consumption by resident SRB (Holowenko et al. 2000; Penner and Foght 2010;

Ramos-Padrón et al. 2011; Stasik et al. 2014). SRB may thrive on a variety of organic compounds including low-molecular weight fatty acids (Stasik et al. 2015; Stasik and Wendt-Potthoff, 2016) and residual hydrocarbons (Tan et al. 2015; Abu Laban et al. 2015; Folwell et al. 2016; Gee et al. 2017) to produce hydrogen sulfide (H_2S) gas. First investigations performed to characterize microorganisms in Syncrude MLSB revealed the domination of SRB over methanogens (Foght et al. 1985). Subsequent studies confirmed the presence of high numbers of SRB in OSTP from different operators: e.g., Syncrude's MLSB (10^2–10^9 dry-g^{-1}) (Sobolewski 1999a, b; Holowenko et al. 2000; Fedorak et al. 2002; Penner and Foght 2010) and WIP (10^3–10^7 mL^{-1}) (Sobolewski 1999a; Penner and Foght 2010; Stasik and Wendt-Potthoff 2014; Stasik et al. 2014), Suncor's Pond 1 (10^1–10^9 dry-g^{-1}) (Sobolewski 1997, 1999a, Fedorak et al. 2002), Shell Albian's Muskeg River Mine (10^5–10^8 mL^{-1}) (Li 2010) and CNRL's Horizon Mine (10^5 mL^{-1}) (Foght and Siddique 2014). As a result of microbial SO_4^{2-} reduction, a distinct sulfidic zone evolved in many OSTP, potentially generating huge quantities of H_2S (MacKinnon 1989; Holowenko et al. 2000; Penner and Foght 2010; Ramos-Padrón et al. 2011; Stasik et al. 2014). Due to the volatility and acute toxicity, H_2S poses a strong concern for aquatic and terrestrial environments near OSTP (BGC Engineering 2010).

For both Suncor's (Pond 6) and Syncrude's (WIP and MLSB) OSTP, increased sulfide (H_2S/HS^-) production in the upper 15 m below the mudline interface was positively correlated with numbers of SRB (e.g., *Desulfocapsa*, *Desulfobacterium*, *Desulfatibacillum*, *Desulfuromonas* spp., and *Desulfurivibrio* spp.) and the highest measured sulfate reduction rates (10–100 μM d^{-1}) (Penner and Foght 2010; Ramos-Padrón et al. 2011; Stasik et al. 2014). However, while high concentrations of soluble sulfide (e.g., up to 3 mM H_2S/HS^- in Suncor's Pond 6) (Ramos-Padrón et al. 2011) and acid-volatile sulfur (AVS) including H2S/HS^- and FeS (e.g., 2.59–5.54 mM in the WIP) (Stasik and Wendt-Potthoff 2014; Stasik et al. 2014) were detected below the water-tailings interface, surface waters had typically low (0–0.52 mM) (Chen et al. 2013) or undetectable H_2S/HS^- (Ramos-Padrón et al. 2011), indicating that H_2S outgassing from the OSTP was limited in situ. These findings were confirmed by the observations from comprehensive bioreactor studies with FFT, where a sulfidic zone composed of H_2S and insoluble iron monosulfide (FeS) evolved along a well-defined redox gradient just below the mudline with little or no H_2S diffusion into the water cap during long-term incubation (Chi Fru et al. 2013; Chen et al. 2013; Liu et al. 2016).

Beside the immobilization of produced H_2S by the primary incorporation into FeS in presence of reactive Fe (HCl soluble and hydroxylamine reducible Fe^{II} and Fe^{III}) (Salloum et al. 2002; Chen et al. 2013; Siddique et al. 2014b; Reid et al. 2016), the further transformation of FeS to the more stable pyrite (FeS_2) was observed to promote the permanent burial of S into the mineral phase of OSTP (Chen et al. 2013; Stasik and Wendt-Potthoff 2014; Stasik et al. 2014; Dompierre et al. 2016). In addition to precipitation, H_2S incubated with tailings in the presence of air underwent a rapid chemical re-oxidation to S and SO_4^{2-}, which likely reflected the high concentrations of SO_4^{2-} (2–6 mM) typically measured in the oxic surface water in OSTP (Ramos-Padrón et al. 2011). Similarly, the reaction of H_2S diffusing

downward with buried Fe oxides and (oxy) hydroxides clay minerals (Kaminsky et al. 2008; Chen et al. 2013) may involve the formation of elemental S which is repeatedly detected in anoxic tailings of the WIP (Stasik and Wendt-Potthoff 2014; Stasik et al. 2014). This process may also explain the presence of a secondary SO_4^{2-} containing zone in deeper layers of Suncor's Pond 6 (Ramos-Padrón et al. 2011).

Apart from the chemical oxidation of H_2S, potential microbial S oxidation (Stasik et al. 2014) and high numbers of S-oxidizing bacteria (SOB) (e.g., 10^4–10^8 mL^{-1}) at different depths in Syncrude's WIP and MLSB further indicate biologically mediated sulfide re-cycling by microbes affiliated with *Thiobacillus* spp. (Penner and Foght 2010). Thus, current investigations suggest that H_2S outgassing from OSTP may be effectively prevented by the chemical and biological re-oxidation of sulfide as well as by a direct coupling of Fe and S cycling within a few meters below the mudline, leading to the immobilization of both toxic H_2S and heavy metals (Fig. 3). Moreover, H_2S exhibits a relatively higher solubility in water which increases even more by the dissociation to HS^- at pH 7–8 typically measured in OSTP (Ramos-Padrón et al. 2011). In line with these findings, surface H_2S emission measured by gas flux chambers from various OSTP are generally low or below the level of detection (Small et al. 2015).

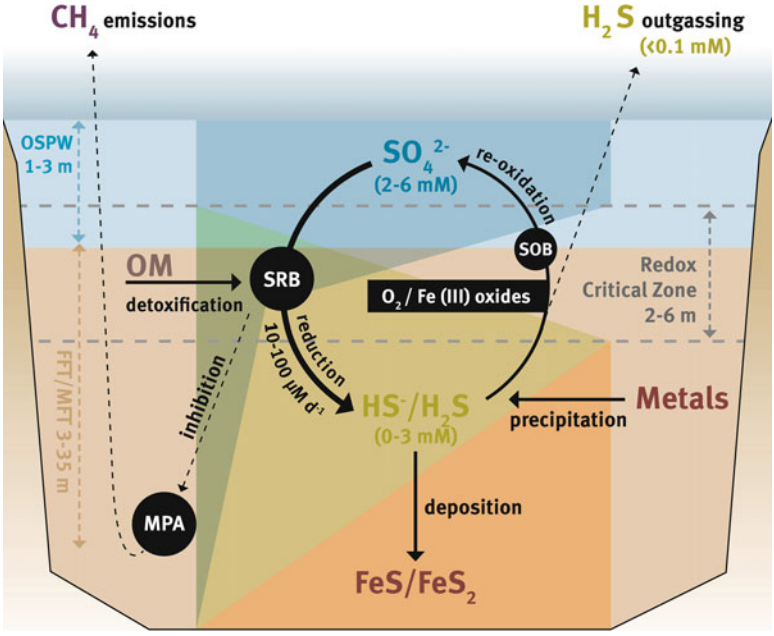

Fig. 3 Schematic illustration summarizing the relevance of S biogeochemistry in OSTP modified from Stasik and Wendt-Potthoff (2016): Mineralization/detoxification of organic matter (OM) by SRB; production/outgassing of toxic and volatile H_2S; H_2S re-oxidation via SOB and/or O_2/$Fe(OH)_3$; H_2S precipitation/metal immobilization and sedimentation; Impact of SO_4^{2-} reduction on CH_4 emissions. *SRB* – sulfate-reducing bacteria; *SOB* – sulfur-oxidizing bacteria; *MPA* – methanogenic archaea

Owing to methanogenesis's implications in tailings densification and GHG emissions, impact of S cycling on CH_4 production has also been investigated in OSTP. While SO_4^{2-} reduction is usually too low to affect methanogenesis in deep OSTP layers due to limited SO_4^{2-} replenishment, both processes presumptively compete for common substrates in SO_4^{2-}-rich tailings just below the mudline (Holowenko et al. 2000; Penner and Foght 2010; Stasik et al. 2014). Thus, in several laboratory incubations, CH_4 production significantly decreased in tailings supplemented with SO_4^{2-} (2–6 mM) (Holowenko et al. 2000; Fedorak et al. 2003; Salloum et al. 2002; Ramos-Padrón et al. 2011), suggesting a partial inhibition of methanogenesis and GHG emissions in tailings by SRB due to the competition for the major fermentation products H_2 and acetate. Indeed based on the free energy available, SRB obtain more energy from the oxidation of the principle methanogenic substrates, hydrogen (H_2) and acetate (CH_3COO^-) (Holland et al. 1987) outcompeting methanogens when SO_4^{2-} is available. In line with these findings, both processes were also shown to operate simultaneously in tailings supplemented with an excess of labile organic substrates (e.g., acetate, propionate, formate, lactate, and butyrate) (Stasik and Wendt-Potthoff 2016), indicating limited common substrates in situ. Extrapolations based on in situ rates and microbial potentials determined in microcosm incubations propose that microbial SO_4^{2-} reduction might prevent CH_4 emission in the range of two–five million L CH_4 day^{-1} from large sized OSTP like Suncor's Pond 6 and Syncrude's WIP (Ramos-Padrón et al. 2011; Stasik and Wendt-Potthoff 2016). Taking into account a daily CH_4 emission of ~40 million L estimated for Syncrude's MLSB (Holowenko et al. 2000), reduction in CH_4 would correspond to 5–12.5% of total CH_4 emission depending on the availability of SO_4^{2-}.

In addition to the utilization of H_2 and low molecular weight fatty acids as metabolites, most SRB identified in OSTP are known to directly degrade organic constituents in tailings and therefore, play an important role in tailings' detoxification. Complex NAs were biodegraded by *Desulfobulbus*, *Desulfomicrobium*, and *Desulfobacterium* under sulfate-reducing condition in an enrichment culture derived from anoxic tailings (Clothier and Gieg 2016). Similarly, complete biodegradation of toluene by members of *Desulfobulbaceae* (Abu Laban et al. 2015) and *n*-alkanes (C_6–C_{10}) by *Desulfobacteraceae*, *Desulfovibrio*, and *Desulfoglaeba* (Tan et al. 2015) in MFT enrichment cultures, and PAH biodegradation by OSPW microbes (Folwell et al. 2016) was observed under sulfate-reducing condition. These studies also revealed fumarate addition as the main activation pathway for organic constituents' metabolism under sulfate-reducing conditions.

Hence, S biogeochemical cycling plays an important role in reducing GHG emissions, immobilizing trace metals, and detoxifying toxic organic constituents in OSTP. Furthermore, the assessment of S stability in composite tailings (CT) reclaimed under wetland (Warren et al. 2016; Reid and Warren 2016) or in EPL (Dompierre et al. 2016) is crucial to determine potential long-term effects of sulfur biogeochemistry on the development of future ecosystems, including the potential generation of acid rock drainage (Kuznetsov et al. 2015, 2016) under upland reclamation scenario.

3.3 Nitrogen Cycling

Nitrogen in OSTP is mostly available as ammonia, e.g., 8–12 mg L^{-1} NH_3-N in Syncrude's WIP (Stasik et al. 2014) and up to 64 mg L^{-1} in Suncor tailings (Fedorak et al. 2002). Nitrogen may also be present in organic form as hetero-atom in some NAs (Barrow et al. 2010). The concentrations of NO_3^- are generally very low (<0.5 mg L^{-1} or below detection limit (Dompierre et al. 2016; Reid et al. 2016)); and given the reduced status of the MFT in OSTP, NO_3^- reduction is unlikely to be a prominent anaerobic process in these ecosystems. Under anaerobic conditions, nitrification is inhibited by both the lack of O_2 and the specific toxicity of NAs to nitrifying organisms in the oxic part of OSPW (Misiti et al. 2013) that may prevent regeneration of NO_3^-. NAs are noninhibitory to denitrifiers up to 400 mg L^{-1} (Misiti et al. 2013). However, while complex acid-extractable organics naturally present in OSPW are not degradable under nitrate-reducing conditions, potential transformation of simple single-ringed surrogate NAs (presumptively via β-oxidation) was observed in enrichment cultures from anoxic tailings under nitrate-reducing conditions (Clothier and Gieg 2016). Thereby, NO_3^- loss directly correlated with a transient production of nitrite (NO_2^-). In these cultures, the most prominent genera were *Terrabacter*, *Derxia*, and *Limnobacter*. Although degradation of oil sands-derived hydrocarbons is possible under nitrate-reducing conditions (Luo et al. 2014; Gunawan et al. 2014; Clothier and Gieg 2016), potential rates of NO_3^- reduction/denitrification have not been determined in OSTP, except for an ex situ approach for the treatment of OSPW in bioreactors, where the highest NO_3^- removal rates (e.g., up to 3164.7 mg L^{-1} h^{-1}) coincided with maximum turnover rates of NAs (Gunawan et al. 2014).

In comparison to organic carbon concentrations, the OSTP are rather poor in N. Therefore, the ability to fix dinitrogen (N_2) could be important to tailings-inhabiting microorganisms. N_2 fixation potential has indeed been detected in methanogenic MFT cultures where member of *Hyphomicrobiaceae* and *Clostridium* in N-deficient cultures metabolized citrate into CH_4, supporting the idea that members of these genera were involved in N_2 fixation (Collins et al. 2016). As another option to meet microbial N requirement, the use of polyacrylamide as a potential N source in OSTP has been tested. Some tailings operators add polyacrylamide to FFT for the purpose of clay flocculation to consolidate tailings. Depending on the polymer and tailings characteristics, 3–30 mg L^{-1} is added to fresh FFT (Vedoy and Soares 2015). Polyacrylamide is not readily available to microbes but may be slowly degraded by combinations of mechanical, photochemical, and biological processes (Guezennec et al. 2015). Polyacrylamide deamination has been reported to provide N to tailings microbes for methanogenesis (Haveroen et al. 2005), but contradictory results revealed that polyacrylamide was not utilized as N source by methanogenic MFT cultures (Collins et al. 2016). These contrasting findings may be due to different source materials and experimental procedures.

Despite low NO_3^- concentrations, most probable number (MPN) counts of nitrate-reducing bacteria have found to be rather high (Table 2). Many bacterial genera from several phyla/classes (*Alpha*-, *Beta*-, and *Gamma-Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Clostridia*, *Bacilli*) include organisms capable of NO_3^-

Table 2 Most probable number estimations of the abundance of nitrate-reducing and iron-reducing prokaryotes

OSTP and sample type	Sample depth (mbs)	Sampling date(s)	Most probable number		References
			Nitrate-reducing prokaryotes	Iron-reducing prokaryotes	
Syncrude MLSB					
Water	<1	1998	10^5/mL	BDL	Sobolewski (1999b)
MFT	5–30	1998	10^2–10^5/dry g	0–10^3/dry g	Sobolewski (1999b)
MFT	9–29	1992	10^3–10^5/mL	10^3–10^4/mL	Sobolewski (1999a)
MFT	7	1998	10^8/dry g	10^2/dry g	Fedorak et al. (2002)
Syncrude West In-Pit					
Water	1.5, 3	2011	ND	10^1–10^2/mL	Stasik et al. (2014)
MFT	7–10	1998	10^2–10^5/dry g	BDL	Sobolewski (1999b)
MFT	4.5–18.5	2011	ND	10^3–10^4/mL	Stasik et al. (2014)
CT	NA	1998	10^4/dry g	10^1/dry g	Fedorak et al. (2002)
Suncor Pond 1					
MFT	7–13	1992	10^3–10^5/mL	10^2–10^3/mL	Sobolewski (1999a)
MFT	3.5	1998	10^9/dry g	10^3/dry g	Fedorak et al. (2002)
CT	NA	1998	10^9/dry g	10^3/dry g	Fedorak et al. (2002)
Albian Sands Muskeg River Mine					
MFT	7	2008	ND	10^3–10^4/mL	Li (2010)
CNRL Horizon Mine					
MFT	8.5	2013	10^8/L	BDL	Foght and Siddique (2014)
MFT	16	2013	10^9/L	BDL	Foght and Siddique (2014)
FFT (fresh)	NA	2013	10^6/L	BDL	Foght and Siddique (2014)

CT consolidated tailings, *MFT* mature fine tailings, *FFT* fluid fine tailings, *ND* not determined, *NA* not applicable, *BDL* below detection limit

reduction. A range of taxa containing this metabolic function have been detected in the core microbiome of OSTP (*Comamonadaceae*, *Acidovorax*, *Rhodoferax*, *Alcaligenaceae*, *Hydrogenophilaceae*, *Thiobacillus*, *Alteromonadaceae*, *Lutibacter*;

Wilson et al. 2016). However, the majority of these microorganisms are facultative nitrate-reducers, which may also use other external electron acceptors or may thrive on fermentation of organic matter. In addition, some of the known nitrate-reducers like clostridia may form endospores, so they have probably been present as spores at the time of sampling. When OSTP are converted to EPL by stopping MFT addition and adding freshwater to increase and dilute the freshwater cap, nitrification may establish, thus completing the N cycle in the lake. This is desirable from two points of view: (1) the regeneration of NO_3^- as an electron acceptor may help degrade residual hydrocarbons and limit H_2S and CH_4 production, and (2) nitrification is needed to establish a healthy ecosystem, as ammonia is toxic for fish above 0.5 mg L^{-1} at slightly alkaline pH.

3.4 Iron Cycling

Total Fe may reach 2–3% of the MFT solid phase mass, and more than half of this comprised Fe^{III} oxide minerals even after prolonged incubation (Siddique et al. 2014b). Bioavailable Fe concentrations in CT (Kingfisher CT deposit, Syncrude) have been found to increase with tailings depth (23–47 μmol g^{-1}; Warren et al. 2016). This fraction includes reducible amorphous and crystalline ferric (Fe^{III}) oxides which can be extracted by combinations of HCl/hydroxylamine and acetic acid/hydroxylamine, respectively (Haack and Warren 2003). Dissolved Fe concentrations can roughly be interpreted as ferrous Fe (Fe^{II}; primarily as Fe^{2+}), as Fe^{III} is practically insoluble at the alkaline pH of oil sands tailings. Low concentrations of dissolved Fe (<0.1–0.6 mg L^{-1}) have been detected in WIP/Base Mine Lake (BML) (Stasik et al. 2014; Dompierre et al. 2016). The presence of dissolved Fe implies that some form of Fe^{III} reduction has already taken place, either as direct microbial/enzymatic reduction or as a side reaction of microbial SO_4^{2-} reduction through chemical reduction by H_2S. Total reactive Fe (the sum of HCl soluble Fe^{II} and hydroxylamine reducible Fe^{III}) in WIP reached 2500–3000 mg L^{-1}, which was again mostly Fe^{II} (Stasik et al. 2014). Given the low dissolved Fe concentrations, a large part of this amount must be Fe^{II} minerals. Various Fe^{II} minerals have been either detected in oil sands tailings or their presence has been inferred through geochemical equilibrium modelling. Among these are FeS, FeS_2, siderite ($FeCO_3$), and vivianite ($Fe_3(PO_4)_2.8H_2O$) (Dompierre et al. 2016; Siddique et al. 2014b). Fe-containing clay minerals, namely, illite and chlorite, have also been detected (Dompierre et al. 2016). Fe^{III} in clay minerals may also be microbially reduced (Kostka et al. 2002; Jaisi et al. 2007; Zhang et al. 2012).

Iron-reducers have been regularly detected in all kinds of OSTP. Their numbers are mostly low and fewer than nitrate-reducers (Table 2). This is surprising given the higher abundance of their external electron acceptor (Fe^{III} minerals) in OSTP and the ability of many known iron-reducers to use alternative electron acceptors (Lovley 2006). However, using available potential Fe^{III} reduction rates and MPN to calculate rates per cell, this gives daily turnover rates in the picomolar range. Compared to rates obtained from cultures of *Shewanella* species or Deltaproteobacteria of 0.48

and 1.44 fmol cell^{-1} day^{-1} (Nealson and Saffarini 1994), these rates are unrealistically high. This means that the true number of iron-reducing organisms has been severely underestimated in OSTP, which has also been found when studying other aquatic habitats (Nielsen et al. 2002; Meier et al. 2005). Potential rates of Fe^{III} reduction have been measured in anaerobic incubations (Stasik et al. 2014) which ranged from <20 nmol mL^{-1} day^{-1} in the water cap to 48–412 nmol mL^{-1} day^{-1}in the MFT of WIP. When synthetic ferric (Fe^{III}) hydroxide was added as a Fe source, the Fe^{III} reduction rates were significantly (several times) higher. This clearly shows that Fe^{III} reduction was limited by the availability of Fe^{III} in WIP. Addition of molybdate to the incubations as a specific inhibitor of SO_4^{2-} reduction and measurements of Fe^{III} reduction rates compared to molybdate-free incubations amended with ferric hydroxide suggest the occurrence of direct microbial Fe^{III} reduction, while Fe^{III} reduction by microbially produced H_2S was of minor importance under in situ conditions.

Regarding the taxonomic affiliation of iron-reducers in OSTP, almost nothing is known so far. Iron-reducers are not taxonomically uniform, as this trait is believed to be very old and may have evolved polyphyletically (Lonergan et al. 1996; Weber et al. 2006). Still, Fe^{III} reduction may be performed by microbes not well known for this ability (e.g., Lehours et al. 2009; Zhang et al. 2012). The definition of a core microbiome for OSTP has revealed several taxa, which contain microbes involved in Fe^{III} reduction (*Comamonadaceae*, *Rhodoferax*, *Rhodocyclaceae*; Wilson et al. 2016). Regarding the electron donors utilized for Fe^{III} reduction in OSTP, partial degradation of NAs is speculated (Clothier and Gieg 2016). Though no research reports hydrocarbon biodegradation under iron-reducing condition in OSTP, the Fe^{III}-driven biodegradation of crude oil hydrocarbons in situ (Bekins et al. 2016) or BTEX under sediment-free iron-reducing conditions (Jahn et al. 2005) has been demonstrated. Therefore, depending on Fe^{III} availability, microbial Fe^{III} reduction can be important for hydrocarbon biodegradation in OSTP.

Fe^{III} minerals may influence CH_4 emissions from OSTP by stimulating anaerobic CH_4 oxidation (Amos et al. 2012) or inhibiting methanogenesis. Several recent studies signify the implications of Fe^{III} minerals in methanogenesis. Reduction of amorphous Fe^{III} minerals such as ferrihydrite ($5Fe_2O_3.9H_2O$) coupled to organic substrate oxidation can inhibit methanogenesis by diverting electron flow from methanogenesis to Fe^{III} reduction and outcompeting methanogenic archaea by iron-reducers (Yamada et al. 2014; Zhou et al. 2014; Bray et al. 2017). Conversely, crystalline Fe^{III} minerals such as goethite (FeOOH) and magnetite ($Fe^{2+}Fe^{3+}{}_2O_4$) enhance methanogenesis facilitating direct inter-species electron transfer (Tang et al. 2016; Bray et al. 2017; Yao et al. 2017; Zheng et al. 2017) or through soluble microbial products (Zhu et al. 2017). Therefore, it is important to comprehensively study CH_4 and Fe cycling in OSTP for its management with respect of GHG emissions, hydrocarbon biodegradation, and clay consolidation (see next Sect. 3.5). Overall, the intensity of Fe cycling in OSTP may be limited by re-oxidation of Fe^{II}. However, as Fe^{II} is chemically oxidized upon contact with O_2, an intense Fe cycle is likely to be established at the mudline in OSTP.

3.5 Biodensification/Consolidation of Suspended Clays in Oil Sands Tailings

Reducing the volume and increasing the density and strength of tailings (consolidation of tailings) are the important challenges (technically and environmentally) for oil sands mining industry. The FFT deposited in tailings ponds settle by gravity in 2–4 years to form MFT that would further require 125–150 years to consolidate ≥60 wt% final solids contents (Eckert et al. 1996) in the absence of flocculant additions or other aides. However, field observations in southern zone of MLSB correlated accelerated consolidation of tailings with a period of intense methanogenic microbial activity and biogenic gas bubbling (Guo 2009; Holowenko et al. 2000). The possible explanation of how microbial activity enhances tailings densification could be the ebullition of biogenic gases (CO_2 and CH_4) potentially creating channels in semi-solid MFT that allow upward migration of pressurized porewater (Voordouw 2012). Brown et al. (2013) endorsed this finding and added that densification of tailings could also occur under nitrate-reducing and sulfate-reducing conditions and that both microbial biomass and gas de-watering channels might be responsible for tailings densification.

A comprehensive biogeochemical model describing possible mechanisms of MFT biodensification was developed by Siddique et al. (2014a, b; Fig. 4) based on a study using Syncrude MFT in 50-L columns that were either unamended or amended with low concentrations of a complex carbon source to accelerate indigenous methanogenic microbial activity. Microbial metabolism changed the chemistry of both porewater and solid fraction (fine mineral) of MFT in several interrelated ways. Ebullition of biogenic gases (CH_4 and CO_2) particularly CH_4 (due to poor solubility of CH_4 in water) in MFT zone near the mudline potentially created transient channels to allow pressurized porewater to escape to the surface. Dissolution of produced CO_2 in MFT lowered porewater pH, dissolved carbonate minerals in MFT, and increased calcium (Ca^{2+}), magnesium (Mg^{2+}) and bicarbonate (HCO_3^-) ions in the porewater (Fig. 4b; pathway I). Greater concentrations of soluble ions increased the ionic strength of porewater and decreased the thickness of the diffuse double layer (DDL) of clay particles by decreasing the charge potential on clays. Decreasing thickness of DDL increases clay flocculation that caused rearrangement of clays from "house of cards" to "deck of cards" structures (Fig. 4a, c) leading to accelerated densification of tailings (Siddique et al. 2014a). Arkell et al. (2015) reported similar observations when effect of methanogenic microbial metabolism on tailings consolidation was assessed at small scale (2-L column experiments). They further revealed that increased concentrations of exchangeable divalent cations, particularly Mg^{2+}, on clay exchange surfaces also contributed to the decrease in DDL thickness in addition to the role of other soluble ions in the porewater. Zhu et al. (2011) used pressurized CO_2 in the densification (abiotic) of oil sands tailings from Syncrude and CNRL and attributed improvement in tailings densification to pH reduction.

Pathway II (Fig. 4b) elucidates the concurrent changes in the solid phase chemistry through biogeochemical processes driven by the indigenous tailings microbes

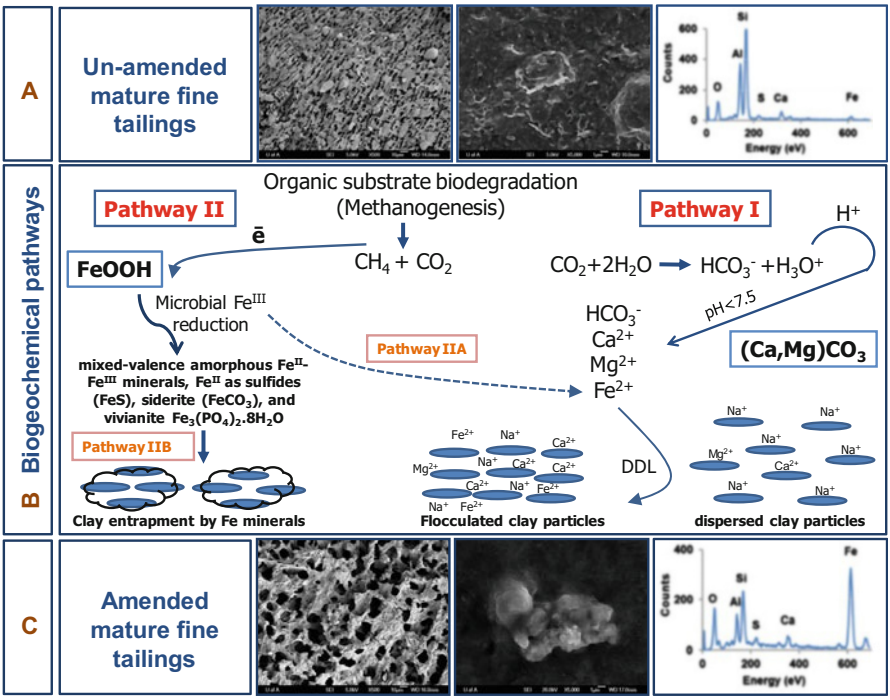

Fig. 4 Proposed microbially driven geochemical pathways of clay consolidation, modified from Siddique et al. 2014b. (**a**) Unamended mature fine tailings (MFT) with dispersed clay particles lacking transformed Fe mineral coating; (**b**) Biogeochemical pathways that were induced when MFT was amended with organic substrate. Pathway I shows how increased anaerobic (methanogenic) microbial activity in amended MFT changes porewater chemistry facilitating tailings consolidation. Pathway II shows transformation of Fe^{III} minerals (FeOOH and $5Fe_2O_3{\cdot}9H_2O$) in amended MFT under methanogenic condition to Fe^{II} as dissolved Fe^{2+} that may contribute to the cation exchange process (pathway IIA) and to formation of mixed-valence Fe^{II}–Fe^{III} and other Fe^{II} minerals (FeS, $FeCO_3$, and $Fe_3(PO_4)_2{\cdot}8H_2O$). Transformed minerals entrap clay particles and/or mask the reactive surfaces of clays, increasing clay particle consolidation (Pathway IIB). Competing reactions are shown by solid arrows, while dashed arrows indicate pathways not considered significant; and (**c**) Amended MFT with flocculated clay particles (coated with amorphous Fe minerals) having card house structure producing a network of interstitial pores facilitating dewatering and consolidation of tailings

to promote biodensification/consolidation (Siddique et al. 2014b). Molecular analysis of microbial communities in MFT suggests that Fe transformation in amended MFT was not carried out by canonical Fe-reducing bacteria, rather methanogenic MFT bacteria (predominantly *Clostridiales*, *Synergistaceae* and *Desulfobulbaceae*) and archaea (predominantly *Methanolinea, Methanoregula* and *Methanosaeta*) transformed crystalline Fe^{III} minerals ($5Fe_2O_3 \cdot 9H_2O$ and FeOOH) to amorphous Fe^{II} minerals (FeS, $Fe_3(PO_4)_2{\cdot}8H_2O$, $FeCO_3$, and possible green rust) during metabolism. *Clostridiales* and *Synergistaceae* ferment organic carbon in amended MFT to produce simple fatty acids, alcohols, CO_2, and H_2 as substrates for SRB such as

Desulfobulbaceae, and for methanogens via syntrophic interactions. Fermenters can divert a portion of the electrons from fermentation to Fe^{III} as an electron sink or supplementary electron acceptor without conserving sufficient energy for cell growth (Coleman et al. 1993; Dobbin et al. 1999). These observations are supported by the findings that *Clostridiales* and *Desulfobulbaceae* were involved in Fe^{III} reduction during benzene degradation (Kunapuli et al. 2007). Jiang et al. (2013) reported syntrophic acetate oxidation by Clostridia and methanogens through Fe^{III}/Fe^{II} cycling. Very recent studies also revealed enhanced methanogenesis during the transformation of Fe^{III} minerals (Zheng et al. 2017; Yao et al. 2017; Bray et al. 2017). Whether *Desulfobulbaceae* in MFT directly reduce Fe^{III} or shuttle electrons to Fe^{III} through S^{2-}/S^0 cycling (Straub and Schink 2004) could not be discerned. However, the role of biogenic S^{2-} in chemical reduction of Fe^{III} minerals is not considered significant because of the low initial concentration of SO_4^{2-} in MFT and small amounts of amorphous FeS formation during incubation (Siddique et al. 2014b). Methanogens that reduce CO_2 using H_2 can also transfer electrons to Fe^{III} either directly or through electron shuttles like extracellular quinones (Bond and Lovley 2002; Liu et al. 2011).

Microbial transformation of Fe^{III} minerals produced Fe^{II} and/or mixed-valence (Fe^{III}/Fe^{II}) minerals that entrapped and masked electronegative clay surfaces and thereby increased their flocculation. Both porewater and solid phase biogeochemical changes aided aggregation of clays and formation of networks of pores (visualized by cryo-scanning electron microscopy; Fig. 4c) that accelerated de-watering and consolidation of MFT. This model has been validated in part by subsequent field observations (Dompierre et al. 2016). Mechanism(s) of "biodensification" (MFT consolidation and de-watering aided by anaerobic microbial activity) might help in developing technologies to manage the large volumes of tailings for subsequent reclamation and recycling of recovered porewater in the bitumen extraction process.

4 Microorganisms and Biogeochemical Processes in Oil Sands Tailings Under Aerobic Condition (Acid Rock Drainage Potential of MFT if Reclaimed Under Aerobic Condition)

Froth flotation tailings are different from other tailings streams containing greater proportions of iron-containing minerals like FeS_2. Their retention as colloidal suspensions in anaerobic ponds below a water layer prevents oxidation of FeS_2. TT are produced by adding polymers to flocculate clays to reduce the volume and increase the shear strength of tailings in preparation for reclamation. Evaporative drying of multiple thin layers of TT on gentle slope for dry reclamation exposes the TT to atmospheric O_2 and natural precipitation (rainwater and snow) that may expedite microbially mediated acid rock drainage (ARD). Froth treatment tailings that are enriched in significant proportions of FeS_2 are particularly prone to the generation of ARD during atmospheric drying. Kuznetsov et al. (2015) exposed two different froth treatment TT samples (TT1; no flocculant added, and TT2; treated

with polyacrylamide as a flocculant) to controlled moisture and airflow in the laboratory, simulating exposure to environmental conditions. Acid-base accounting (ABA) of the initial TTs samples, calculated based on the contents of FeS_2 (an acid producing mineral) and carbonates (alkaline minerals) (Fig. 5a), showed that both TT1 and TT2 initially had net acid-producing potential. TT2 having greater acid producing potential (ABA value of −230 t $CaCO_3$ equiv. 1000 t^{-1} of TT) irrigated with distilled water (TT-DW) exhibited acid rock drainage within 50 days, producing a leachate of pH ≤2. TT1 (ABA value of −141 $CaCO_3$ equiv. 1000 t^{-1} of TT) irrigated with artificial rainwater (TT-AR) started generating acidic leachate by 250 days (Fig. 5b).

Pyrosequencing of 16S rRNA gene analyses of periodical sampling during the experiment (Fig. 5c) revealed that the initial TT microbial communities comprised

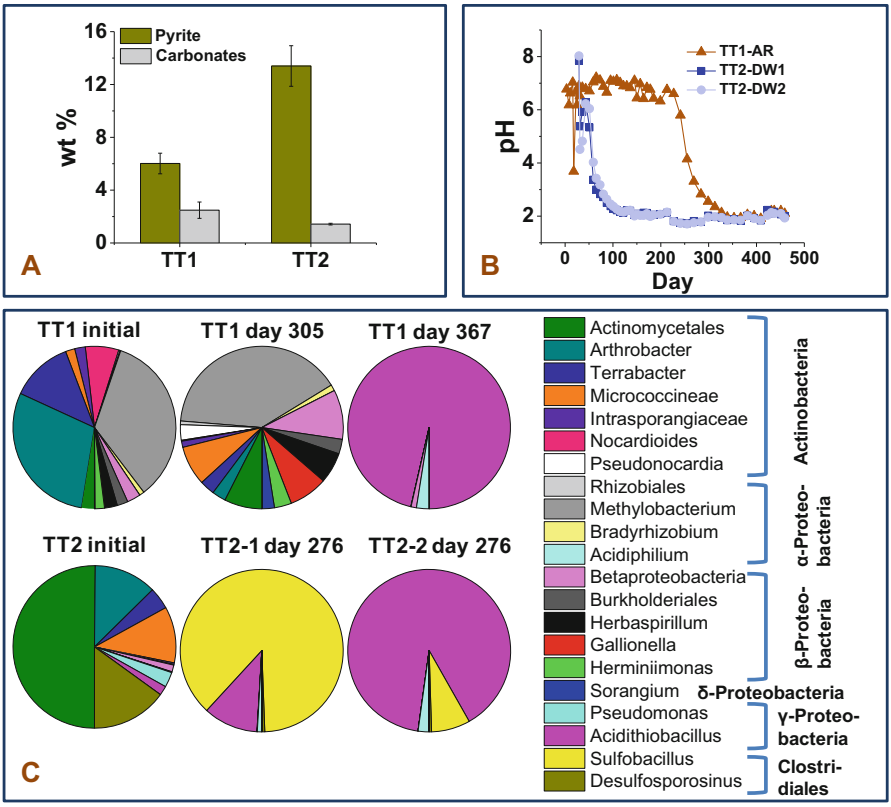

Fig. 5 Acid rock drainage from thickened tailings (TT) during atmospheric drying. (Modified from Kuznetsov et al. 2015). (**a**) Pyrite and carbonate minerals contents in initial TT (TT1; no flocculant added and irrigated by artificial rain water [TT1-AR], and TT2; treated with polyacrylamide as a flocculant and irrigated by distilled water [TT2-DW]), (**b**) pH of leachates collected from TT incubated in irrigation trays, and (**c**) microbial community structure determined through 16S rRNA gene pyrotag sequencing of DNA extracted from TT samples collected over time during the incubation

diverse members of the bacterial taxa Actinobacteria (*Actinomycetales*), Proteobacteria (*Alpha-*, and *Beta-Proteobacteria*), and Firmicutes (*Clostridiales*). A rapid shift in microbial community composition was observed in TTs when conditions became strongly acidic (pH ~ 2) that favored the enrichment of indigenous S- and/or Fe-oxidizing bacteria: *Acidithiobacillus* and *Sulfobacillus*. Both *Sulfobacillus* and *Acidithiobacillus* oxidize pyrite (Xingyu et al. 2009; Pina et al. 2010; Wang et al. 2014). The dominance of these bacterial strains strongly implicates them in the development of ARD (Kuznetsov et al. 2015). Dean et al. (2016) used TT samples from the same source for the isolation of microorganisms from cultivable enrichment cultures grown at pH 7, 4.5 and 2.5. The S-oxidizing microorganisms identified were closely related to *Halothiobacillus*, *Achromobacter*, and *Curtobacterium* spp., while *Chitinophagaceae* and *Acidocella* spp. were identified as potential Fe-oxidizing/reducing microbes. Besides acidification, a major consequence of ARD is the increased toxic metals in the leachate/drainage water. Kuznetsova et al. (2016) analyzed the acidic leachates described above and found that soluble metal concentrations reached 10,000 ppb for Ni, 5000 ppb for Co, 3000 ppb for As, 2000 ppb for V, and 1000 ppb for Cr. Therefore, using TT containing higher sulfides directly in dry reclamation scenarios or for tailings volume reduction may unfavorably impact the environment and warrants scrutiny of appropriate strategies to manage FeS_2-enriched oil sands tailings streams, probably in EPL where MFT is retained in anoxic environment under a thick water layer.

5 Microorganisms in the Process-Affected Water Capping Oil Sands Tailings in Ponds

The OSPW layer capping tailings in ponds may vary from 2 to 0 m deep depending on the removal rate from tailings ponds. Because the OSPW layer is constantly being recycled for bitumen extraction and also experiences seasonal changes, a few studies have examined the diversity of OSPW microbiota compared to MFT that lies underneath OSPW (cap water), often undisturbed for decades, enabling long-term microbial studies. OSPW is alkaline (pH 7.7–8.8) and recycling concentrates soluble cations/anions, polycyclic aromatic hydrocarbons, and NAs, a primary source of toxicity in OSPW (Madill et al. 1999; Allen 2008). Fugitive extraction diluent (naphtha or paraffinic solvent containing light hydrocarbons), H_2S, and CH_4 arising from MFT beneath the OSPW impose additional O_2 demand dictating redox conditions and pose toxic pressures on indigenous microbes or support microbial metabolism in cap water zone. The uppermost surface layer of OSPW (roughly 1 m deep) in tailings ponds is oxic due to wind or wave action and conditions become anoxic below the surface and deeper (Ramos-Padrón et al. 2011). Presence of aerobic thiosulfate-oxidizing microbes, abundance of facultative anaerobes such as heterotrophic nitrate-reducers, and lower estimates of iron-reducers and other anaerobes (methanogens and sulfate-reducers) have been reported in Syncrude OSPW (reviewed by Foght et al. 2017).

Using molecular biological approaches such as pyrotag sequencing of 16S rRNA genes, Ramos-Padrón et al. (2011) assessed the prokaryotic community composition at different tailings depth in Suncor Pond 6 and found taxa (*Methyloversatilis*, *Azospirillum*, and *Gemmata*) in surface water that were entirely different from strictly anaerobic taxa identified in deeper tailings strata. Subsequently, An et al. (2013a) found *Rhodocyclales (Thauera), Burkholderiales* (*Acidovorax, Hydrogenophaga, Alcaligenaceae*), and *Flavobacteriales* (*Flavobacterium*) in 15 OSPW samples from three ponds different from MFT taxa from these ponds. Each OSPW taxon includes facultative species that might be suited to the sharp oxycline of the OSPW layer. S oxidizers such as *Chromatiales (Thiocapsa)* and *Desulfuromonadales (Geobacter)* were also prevalent, whereas both methanogens and sulfate-reducers were minor members of OSPW communities as expected (An et al. 2013a).

Presence of S oxidizing bacteria in upper oxic OSPW layer signifies their role in S metabolism and cycling and reduction in H_2S emissions from tailings ponds. Flux of CH_4 from tailings ponds might also be impacted by the existence of oxic OSPW layer at the surface of tailings ponds. In-depth analysis of pyrotag data, microarrays, and a metagenome revealed the presence of aerobic methanotrophic bacteria (predominantly *Methylocaldum*) and characteristic particulate monooxygenase (*pmo*) genes in OSPW (Saidi-Mehrabad et al. 2013). Oxidation of CH_4 to CO_2 by methanotrophs in OSPW suggests mitigation of some of the OSTP GHG emissions. Extrapolation from conservative oxidation rates and OSTP surface areas estimated that ~17% of biogenic CH_4 at the OSTP surface might be oxidized by methanotrophs in the surface water layer, depending upon the residence time of rapidly released CH_4 bubbles and dissolved O_2 in OSPW layers (Saidi-Mehrabad et al. 2013). During winter months when ice covers much of the ponds preventing any mass transfer of atmospheric O_2, aerobic biodegradation would be slower due to depletion of O_2 in the insulated oxic OSPW layer.

Aerobic microbial processes are likely to occur in the uppermost OSPW layer when wind and wave action aerate the surface water during the summer. In situ aerobic biodegradation theoretically would alleviate some of the acute toxicity of OSPW by consuming organic contaminants such as diluent hydrocarbons and mono- and polycyclic aromatic hydrocarbons which would reduce volatile organic carbon emissions from the ponds (Simpson et al. 2010; Small et al. 2015). Evidence supporting this potential activity was provided by An et al. (2013a) who detected genes encoding putative mono- and dioxygenases and O_2-dependent ring cleavage enzymes in the metagenome of OSTP surface waters, showing a diversity of putative hydrocarbon degradation mechanisms. Many of the bacterial genera detected in surface OSPW including *Brevundimonas* (*Caulobacterales*), *Methylocaldum* (*Methylococcales*), *Xanthobacter* (*Rhizobiales*), *Flavobacterium* (*Flavobacteriales*), and diverse members of the order *Burkholderiales* are known to have potential for aerobic hydrocarbon biodegradation (An et al. 2013a; Saidi-Mehrabad et al. 2013). These findings were further supported by Rochman et al. (2017) who employed metagenomics combined with stable isotope probing of 16S rRNA genes and found that *Methyloversatilis* and *Zavarzinia* were the main benzene degraders and the strains of the family *Chromaticaceae* and the genus *Thauera* were the main

naphthalene degraders in benzene and naphthalene spiked surface (0–10 cm) OSPW collected from Syncrude WIP, lately known as BML. Other than hydrocarbons, NAs are the primary source of OSPW toxicity due to their high solubility (Frank et al. 2009 and Rogers et al. 2002). NAs are also natural components of fossil fuels, and complex structures of NAs formed during biodegradation of alicyclic (naphthenes) and highly branched aliphatic hydrocarbons from bitumen resist further biodegradation (Quagraine et al. 2005). NAs are released into the tailings during bitumen extraction from oil sands ore, and they may be generated in tailings ponds as a result of natural biodegradation of unrecovered bitumen in MFT (Quagraine et al. 2005). Many studies focused on their biodegradation under aerobic and anaerobic conditions using indigenous tailings/OSPW microorganisms. In general, most simple surrogate NAs (commercially available) were readily biodegradable; however, the microorganisms were only partially able to consume the NAs naturally present in the tailings and OSPW (Scott et al. 2005; Demeter et al. 2014; Brown and Ulrich 2015; Yue et al. 2015; Clothier and Gieg 2016; Folwell et al. 2016).

6 Conclusions and Research Needs

Considerable research progress has been made to gain insight into microbial processes in OSTP that produce biogenic gases, biodegrade certain organic constituents, and change porewater and solid phase chemistry. These biogeochemical processes can be modeled to predict GHG emissions and FFT consolidation in OSTP. Further research is needed in several OSTP areas, particularly the potential for in situ biodegradation of recalcitrant hydrocarbons such as cycloalkanes and complex petroleum components (e.g., polycyclic aromatic hydrocarbons and resins) under different redox condition in OSTP. Another important aspect is to determine the role of Fe minerals in carbon biotransformations that can affect GHG emissions from OSTP. Establishment of EPLs is considered a viable wet reclamation scenario, and the first field demonstration to assess the feasibility of EPL has recently begun at Base Mine Lake. Biogeochemical processes in EPL are anticipated to strongly influence the success and sustainability of EPLs. Therefore, considerable effort is needed to help predict (and possibly enhance or mitigate) microbial effects in EPLs, primarily rate and duration of legacy biogenic GHG (CH_4 and CO_2) production from residual recalcitrant organics in tailings and potential flux of contaminants (organic and inorganic such as trace metals) from underlying MFT to overlying cap water with the ebullition of biogenenic gases.

Acknowledgments The authors greatly acknowledge the Helmholtz-Alberta Initiative, NSERC, Canada Foundation for Innovation, Alberta Innovates Energy and Environment Solutions, the Institute for Oil Sands Innovation, Syncrude Canada Ltd. Shell Albian Sands, Canadian Natural Resources Ltd., Suncor Energy, and Total E&P Canada for research funding, resource materials and research infrastructure. We thank our many research leaders, collaborators, and co-authors, including Julia Foght, Phil Fedorak, Rejendar Gupta, Ania Ulrich, Christopher Weisener and Camilla Nesbø; industry advisors, particularly Mike MacKinnon and Tara Penner (Syncrude); André Sobolewski (Microbial Technologies) for providing insight and information from early studies;

and Zvonko Burkus (Alberta Environment and Sustainable Resource Development) for valuable discussions. Finally, we gratefully acknowledge the contributions of Kathy Semple, Alsu Kuznetsova, Petr Kuznetsov, Carmen Li, Matthias Koschorreck and invaluable laboratory members too numerous to list who have been essential to the authors' oil sands research.

References

Abu Laban N, Dao A, Foght J (2015) DNA stable-isotope probing of oil sands tailings pond enrichment cultures reveals different key players for toluene degradation under methanogenic and sulfidogenic conditions. FEMS Microbiol Ecol 91:fiv039

Allen EW (2008) Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. J Environ Eng Sci 7:123–138

Amos RT, Bekins BA, Cozzarelli IM et al (2012) Evidence for iron-mediated anaerobic methane oxidation in a crude oil-contaminated aquifer. Geobiology 10:506–517

An D, Caffrey SM, Soh J et al (2013a) Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. Environ Sci Technol 47:10708–10717

An D, Brown D, Chatterjee I et al (2013b) Microbial community and potential functional gene diversity involved in anaerobic hydrocarbon degradation and methanogenesis in an oil sands tailings pond. Genome 56:612–618

Arkell N, Kuznetsov P, Kuznetsova A et al (2015) Microbial metabolism alters pore water chemistry and increases consolidation of oil sands tailings. J Environ Qual 44:145–153

Barrow MP, Witt M, Headley JV, Peru KM (2010) Athabasca oil sands process water: characterization by atmospheric pressure photoionization and electrospray ionization Fourier Transform Ion Cyclotron resonance mass spectrometry. Anal Chem 82:3727–3735

Bekins BA, Cozzarelli IM, Erickson ML, Steenson RA, Thorn KA (2016) Crude oil metabolites in groundwater at two spill sites. Groundwater 54:681–691

BGC Engineering Inc (2010) Oil sands tailings technology review. Oil Sands Research and Information Network UoA, Edmonton, AB, OSRIN Report No. TR-1, pp 136

Boll M, Heider J (2010) Anaerobic degradation of hydrocarbons: mechanisms of C-H-bond activation in the absence of oxygen. In: Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 1011–1024

Bond DR, Lovley DR (2002) Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinones. Environ Microbiol 4:115–124

Bordenave S, Kostenko V, Dutkoski M et al (2010) Relation between the activity of anaerobic microbial populations in oil sands tailings ponds and the sedimentation of tailings. Chemosphere 81:663–668

Bray MS, Wu J, Reed BC et al (2017) Shifting microbial communities sustain multiyear iron reduction and methanogenesis in ferruginous sediment incubations. Geobiology 15:678–689

Brown LD, Ulrich A (2015) Oil sands naphthenic acids: a review of properties, measurement, and treatment. Chemosphere 127:276–290

Brown D, Ramos-Padrón E, Gieg L et al (2013) Effect of calcium ions and anaerobic microbial activity on sedimentation of oil sands tailings. Int Biodeterior Biodegrad 81:9–16

Burkus Z, Wheler J, Pletcher S (2014) GHG emissions from oil sands tailings ponds: overview and modeling based on fermentable substrates. Alberta Environment and Sustainable Resource Development. Electronic report available at http://hdl.handle.net/10402/era.30197:24, pp 2014. Last accessed Dec 2017

Callaghan AV (2013) Enzymes involved in the anaerobic oxidation of *n*-alkanes: from methane to long-chain paraffins. Front Microbiol 4:89

Callaghan AV, Gieg LM, Kropp KG et al (2006) Comparison of mechanisms of alkane metabolism under sulfate-reducing conditions among two bacterial isolates and a bacterial consortium. Appl Environ Microbiol 72:4274–4282

Chalaturnyk RJ, Scott JD, Özüm B (2002) Management of oil sands tailings. Pet Sci Technol 20:1025–1046

Chen M, Walshe G, Chi Fru E et al (2013) Microcosm assessment of the biogeochemical development of sulfur and oxygen in oil sands fluid fine tailings. Appl Geochem 37:1–11

Cheng L, Ding C, Li Q et al (2013) DNA-SIP reveals that Syntrophaceae play an important role in methanogenic hexadecane degradation. PLoS One 8:e66784

Chi Fru E, Chen M, Walshe G et al (2013) Bioreactor studies predict whole microbial population dynamics in oil sands tailings ponds. Appl Microbiol Biotechnol 97:3215–3224

Clothier LN, Gieg LM (2016) Anaerobic biodegradation of surrogate naphthenic acids. Water Res 90:156–166

Coleman ML, Hedrick DB, Lovley DR et al (1993) Reduction of Fe(III) in sediments by sulphate-reducing bacteria. Nature 361:436–438

Collins CEV, Foght JM, Siddique T (2016) Co-occurrence of methanogenesis and N_2 fixation in oil sands tailings. Sci Total Environ 565:306–312

Dean C, Xiao Y, Roberts DJ (2016) Enriching acid rock drainage related microbial communities from surface-deposited oil sands tailings. Can J Microbiol 62:870–879

Demeter MA, Lemire J, George I et al (2014) Harnessing oil sands microbial communities for use in ex situ naphthenic acid bioremediation. Chemosphere 97:78–85

Dobbin PS, Carter JP, Juan CG-S et al (1999) Dissimilatory Fe(III) reduction by *Clostridium beijerinckii* isolated from freshwater sediment using Fe(III) maltol enrichment. FEMS Microbiol Lett 176:131–138

Dompierre KA, Lindsay MBJ, Cruz-Hernandez P et al (2016) Initial geochemical characteristics of fluid fine tailings in an oil sands end pit lake. Sci Total Environ 556:196–206

Eckert WF, Masliyah JH, Gray MR et al (1996) Prediction of sedimentation and consolidation of fine tails. AIChE J 42:960–972

Fedorak PM, Coy DL, Salloum MJ, Dudas MJ (2002) Methanogenic potential of tailings samples from oil sands extraction plants. Can J Microbiol 48:21–33

Fedorak PM, Coy DL, Dudas MJ et al (2003) Microbially-mediated fugitive gas production from oil sands tailings and increased tailings densification rates. J Environ Eng Sci 2:199–211

Foght JM (2008) Anaerobic biodegradation of aromatic hydrocarbons: pathways and prospects. J Mol Microbiol Biotechnol 15:93–120

Foght JM, Siddique T (2014) Microbial analysis of CNRL tailings. Report to Canadian Natural Resources Ltd, Aug 2014

Foght JM, Fedorak PM, Westlake DWS (1985) Microbial content and metabolic activities in Syncrude tailings pond. AOSTRA J Res 1:139–146

Foght JM, Gieg LM, Siddique T (2017) The microbiology of oil sands tailings: past, present, future. FEMS Microbiol Ecol 93:fix034

Folwell BD, McGenity TJ, Price A et al (2016) Exploring the capacity for anaerobic biodegradation of polycyclic aromatic hydrocarbons and naphthenic acids by microbes from oil-sands-process-affected waters. Int Biodeterior Biodegrad 108:214–221

Fowler SJ, Gutierrez-Zamora M-L, Manefield M, Gieg LM (2014) Identification of toluene degraders in a methanogenic enrichment culture. FEMS Microbiol Ecol 89:625–636

Frank RA, Fischer K, Kavanagh R et al (2009) Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. Environ Sci Technol 43:266–271

Gee KF, Yin Poon H, Hashisho Z, Ulrich AC (2017) Effect of naphtha diluent on greenhouse gases and reduced sulfur compounds emissions from oil sands tailings. Sci Total Environ 598:916–924

Gray ND, Sherry A, Grant RJ et al (2011) The quantitative significance of *Syntrophaceae* and syntrophic partnerships in methanogenic degradation of crude oil alkanes. Environ Microbiol 13:2957–2975

Grundmann O, Behrends A, Rabus R et al (2008) Genes encoding the candidate enzyme for anaerobic activation of *n*-alkanes in the denitrifying bacterium, strain HxN1. Environ Microbiol 10:376–385

Guezennec AG, Michel C, Bru K et al (2015) Transfer and degradation of polyacrylamide-based flocculants in hydrosystems: a review. Environ Sci Pollut Res 22:6390–6406
Gunawan Y, Nemati M, Dalai A (2014) Biodegradation of a surrogate naphthenic acid under denitrifying conditions. Water Res 51:11–24
Guo C (2009) Rapid densification of the oil sands mature fine tailings (MFT) by microbial activity. PhD thesis, University of Alberta, Edmonton
Haack EA, Warren LA (2003) Biofilm hydrous manganese oxyhydroxides and metal dynamics in acid rock drainage. Environ Sci Technol 37:4138–4147
Han X, Scott AC, Fedorak PM et al (2008) Influence of molecular structure on the biodegradability of naphthenic acids. Environ Sci Technol 42:1290–1295
Haveroen ME, MacKinnon MD, Fedorak PM (2005) Polyacrylamide added as a nitrogen source stimulates methanogenesis in consortia from various wastewaters. Water Res 39:3333–3341
Headley JV, Peru KM, Tanapat S, Putz G (2002) Biodegradation kinetics of geometric isomers of model naphthenic acids in Athabasca River water. Can Water Res J 27:25–42
Heider J, Schuhle K (2013) Anaerobic biodegradation of hydrocarbons including methane. In: Rosenberg E et al (eds) The prokaryotes-prokaryotic physiology and biochemistry. Springer, Heidelberg, pp 605–634
Holland KT, Knapp JS, Shoesmith JG (1987) Anaerobic bacteria. Chapman and Hall, New York, pp 130–133
Holowenko FM, MacKinnon MD, Fedorak PM (2000) Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. Can J Microbiol 46:927–937
Jahn MK, Haderlein SB, Meckenstock RU (2005) Anaerobic degradation of benzene, toluene, ethylbenzene, and o-xylene in sediment-free iron-reducing enrichment cultures. Appl Environ Microbiol 71:3355–3358
Jaisi DP, Dong H, Liu C (2007) Influence of biogenic Fe(II) on the extent of microbial reduction of Fe(III) in clay minerals nontronite, illite, and chlorite. Geochim Cosmochim Acta 71:1145–1158
Jiang S, Park S, Yoon Y et al (2013) Methanogenesis facilitated by geobiochemical iron cycle in a novel syntrophic methanogenic microbial community. Environ Sci Technol 47:10078–10084
Johnson RJ, Smith BE, Sutton PA et al (2011) Microbial biodegradation of aromatic alkanoic naphthenic acids is affected by the degree of alkyl side chain branching. ISME J 5:486–496
Kaminsky HAW, Etsell TH, Ivey DG, Omotoso O (2008) Characterization of heavy minerals in the Athabasca oil sands. Miner Eng 21:264–271
Kostka JE, Dalton DD, Skelton H et al (2002) Growth of iron(III)-reducing bacteria on clay minerals as the sole electron acceptor and comparison of growth yields on a variety of oxidized iron forms. Appl Environ Microbiol 68:6256–6262
Kunapuli U, Lueders T, Meckenstock RU (2007) The use of stable isotope probing to identify key iron-reducing microorganisms involved in anaerobic benzene degradation. ISME J 1:643–653
Kuznetsov P, Kuznetsova A, Foght JM et al (2015) Oil sands thickened froth treatment tailings exhibit acid rock drainage potential during evaporative drying. Sci Total Environ 505:1–10
Kuznetsova A, Kuznetsov P, Foght JM et al (2016) Trace metal mobilization from oil sands froth treatment thickened tailings exhibiting acid rock drainage. Sci Total Environ 571:699–710
Larter SR, Head IM (2014) Oil sands and heavy oil: origin and exploitation. Elements 10:277–283
Lehours AC, Batisson I, Guedon A, Mailhot G, Fonty G (2009) Diversity of culturable bacteria, from the anaerobic zone of the meromictic Lake Pavin, able to perform dissimilatory-iron Reduction in different in vitro conditions. Geomicrobiol J 26:212–223
Li C (2010) Methanogenesis in oil sands tailings: an analysis of the microbial community involved and its effects on tailings densification. MSc thesis, University of Alberta, Edmonton
Liang B, Wang L-Y, Mbadinga SM et al (2015) *Anaerolineaceae* and *Methanosaeta* turned to be the dominant microorganisms in alkanes-dependent methanogenic culture after long-term of incubation. AMB Express 5:37
Liu D, Wang H, Dong H et al (2011) Mineral transformations associated with goethite reduction by *Methanosarcina barkeri*. Chem Geol 288:53–60

Liu H, Tan S, Yu T, Liu Y (2016) Sulfate reducing bacterial community and in situ activity in mature fine tailings analyzed by real time qPCR and microsensor. J Environ Sci 44:141–147

Lonergan DJ, Jenter HL, Coates JD et al (1996) Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J Bacteriol 178:2402–2408

Lovley DR (2006) Dissimilatory Fe(III)- and Mn(IV)-reducing prokaryotes. Chapter 1.21, Prokaryotes 2:635–658

Luo F, Gitiafroz R, Devine CE et al (2014) Metatranscriptome of an anaerobic benzene-degrading, nitrate-reducing enrichment culture reveals involvement of carboxylation in benzene ring activation. Appl Environ Microbiol 80:4095–4107

MacKinnon MD (1989) Development of the tailings pond at Syncrude's oil sands plant: 1978–1987. AOSTRA J Res 5:109–133

Madill REA, Brownlee BG, Josephy PD, Bunce NJ (1999) Comparison of the ames Salmonella assay and mutatox genotoxicity assay for assessing the mutagenicity of polycyclic aromatic compounds in porewater from Athabasca oil sands mature fine tailings. Environ Sci Technol 33:2510–2516

Mbadinga SM, Wang L, Zhou L et al (2011) Microbial communities involved in anaerobic degradation of alkanes. Int Biodeterior Biodegrad 65:1–13

Meier J, Costa R, Smalla K, Boehrer B, Wendt-Potthoff K (2005) Temperature dependence of Fe(III) and sulfate reduction rates and its effect on growth and composition of bacterial enrichments from an acidic pit lake neutralization experiment. Geobiology 3:261–274

Misiti T, Tandukar M, Tezel U, Pavlostathis SG (2013) Inhibition and biotransformation potential of naphthenic acids under different electron accepting conditions. Water Res 47:406–418

Mohamad Shahimin MF, Siddique T (2017a) Methanogenic biodegradation of paraffinic solvent hydrocarbons in two different oil sands tailings. Sci Total Environ 583:115–122

Mohamad Shahimin MF, Siddique T (2017b) Sequential biodegradation of complex naphtha hydrocarbons under methanogenic conditions in two different oil sands tailings. Environ Pollut 221:398–406

Mohamad Shahimin MF, Foght JM, Siddique T (2016) Preferential methanogenic biodegradation of short-chain n-alkanes by microbial communities from two different oil sands tailings ponds. Sci Total Environ 553:250–257

Nealson KH, Saffarini D (1994) Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. Annu Rev Microbiol 48:311–343

Nielsen JL, Juretschko S, Wagner M, Nielsen PH (2002) Abundance and phylogenetic affiliation of iron reducers in activated sludge as assessed by fluorescence in situ hybridization and micro-autoradiography. Appl Environ Microbiol 68:4629–4636

Penner TJ, Foght JM (2010) Mature fine tailings from oil sands processing harbor diverse methanogenic communities. Can J Microbiol 56:459–470

Pina PS, Oliveira VA, Cruz FLS, Leão VA (2010) Kinetics of ferrous iron oxidation by Sulfobacillus thermosulfidooxidans. Biochem Eng J 51:194–197

Quagraine EK, Headley JV, Peterson HG (2005) Is biodegradation of bitumen a source of recalcitrant naphthenic acid mixtures in oil sands tailings pond waters? J Environ Sci Health Pt A 40:671–684

Rabus R, Wilkes H, Behrends A et al (2001) Anaerobic initial reaction of *n*-alkanes in a denitrifying bacterium: evidence for (1-methylpentyl)succinate as initial product and for involvement of an organic radical in *n*-hexane metabolism. J Bacteriol 183:1707–1715

Ramos-Padrón E, Bordenave S, Lin S et al (2011) Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. Environ Sci Technol 45:439–446

Reid ML, Warren LA (2016) S reactivity of an oil sands composite tailings deposit undergoing reclamation wetland construction. J Environ Manag 166:321–329

Reid T, Boudens R, Ciborowski JJH, Weisener CG (2016) Physicochemical gradients, diffusive flux, and sediment oxygen demand within oil sands tailings materials from Alberta, Canada. Appl Geochem 75:90–99

Rochman FF, Sheremet A, Tamas I et al (2017) Benzene and naphthalene degrading bacterial communities in an oil sands tailings pond. Front Microbiol 8:1845

Rogers VV, Wickstrom M, Liber K, Mackinnon MD (2002) Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Toxicol Sci 66:347–355

Saidi-Mehrabad A, He Z, Tamas I et al (2013) Methanotrophic bacteria in oil sands tailings ponds of northern Alberta. ISME J 7:908–921

Salloum MJ, Dudas MJ, Fedorak PM (2002) Microbial reduction of amended sulfate in anaerobic mature fine tailings from oil sand. Waste Manag Res 20:162–171

Scott AC, MacKinnon MD, Fedorak P (2005) Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. Environ Sci Technol 39:8388–8394

Siddique T, Fedorak PM, Foght JM (2006) Biodegradation of short-chain n-alkanes in oil sands tailings under methanogenic conditions. Environ Sci Technol 40:5459–5464

Siddique T, Fedorak PM, MacKinnon MD, Foght JM (2007) Metabolism of BTEX and naphtha compounds to methane in oil sands tailings. Environ Sci Technol 41:2350–2356

Siddique T, Gupta R, Fedorak PM et al (2008) A first approximation kinetic model to predict methane generation from an oil sands tailings settling basin. Chemosphere 72:1573–1580

Siddique T, Penner T, Semple K, Foght JM (2011) Anaerobic biodegradation of longer-chain *n*-alkanes coupled to methane production in oil sands tailings. Environ Sci Technol 45:5892–5899

Siddique T, Penner T, Klassen J, Nesbø C, Foght JM (2012) Microbial communities involved in methane production from hydrocarbons in oil sands tailings. Environ Sci Technol 46:9802–9810

Siddique T, Kuznetsov P, Kuznetsova A, Arkell N, Young R, Li C, Guigard S, Underwookd E, Foght JM (2014a) Microbially-accelerated consolidation of oil sands tailings. Pathway I: changes in porewater chemistry. Front Microbiol 5:106

Siddique T, Kuznetsov P, Kuznetsov A, Li C, Young R, Arocena JM, Foght JM (2014b) Microbially-accelerated consolidation of oil sand tailings. Pathway II: solid phase biogeochemistry. Front Microbiol 5:107

Siddique T, Mohamad Shahimin MF, Zamir S, Semple K, Li C, Foght JM (2015) Long-term incubation reveals methanogenic biodegradation of C_5 and C_6 *iso*-alkanes in oil sands tailings. Environ Sci Technol 49:14732–14739

Simpson IJ, Blake NJ, Barletta B et al (2010) Characterization of trace gases measured over Alberta oil sands mining operations: 76 speciated C_2-C_{10} volatile organic compounds (VOCs), CO_2, CH_4, CO, NO, NO_2, NOy, O_3 and SO_2. Atmos Chem Phys 10:11931–11954

Small CC, Cho S, Hashisho Z et al (2015) Emissions from oil sands tailings ponds: review of tailings pond parameters and emission estimates. J Pet Sci Eng 127:490–501

Smith BE, Lewis CA, Belt ST et al (2008) Effects of alkyl chain branching on the biotransformation of naphthenic acids. Environ Sci Technol 42:9323–9328

Sobolewski A (1997) Anaerobic microbial populations at the Syncrude lease. Consultant's memo to Syncrude, pp 4

Sobolewski A (1999a) Evolution of microbial populations in process-affected aquatic ecosystems. Consultant's report to Syncrude, Edmonton, June, 49 pp

Sobolewski A (1999b) Survey of anaerobic bacteria in tailings samples at the Syncrude lease. 290 Consultant's report to Syncrude, June, 20 pp

Stasik S, Wendt-Potthoff K (2014) Interaction of microbial sulphate reduction and methanogenesis in oil sands tailings ponds. Chemosphere 103:59–66

Stasik S, Wendt-Potthoff K (2016) Vertical gradients in carbon flow and methane production in a sulfate-rich oil sands tailings pond. Water Res 106:223–231

Stasik S, Loick N, Knöller K, Weisener CG, Wendt-Potthoff K (2014) Understanding biogeochemical gradients of sulfur, iron and carbon in an oil sands tailings pond. Chem Geol 382:44–53

Stasik S, Wick LY, Wendt-Potthoff K (2015) Anaerobic BTEX degradation in oil sands tailings ponds: impact of labile organic carbon and sulfate-reducing bacteria. Chemosphere 138:133–139

Straub KL, Schink B (2004) Ferrihydrite-dependent growth of Sulfurospirillum deleyianum through electron transfer via sulfur cycling. Appl Environ Microbiol 70:5744–5749

Tan B, Dong X, Sensen CW, Foght JM (2013) Metagenomic analysis of an anaerobic alkane-degrading microbial culture: potential hydrocarbon-activating pathways and inferred roles of community members. Genome 56:599–511

Tan B, Rozycki T, Foght JM (2014a) Draft genome sequences of three *Smithella* spp. obtained from a methanogenic alkane-degrading culture and oil field produced water. Genome Announc 2: e01085–e01014

Tan B, Charchuk R, Li C, Laban NA, Foght JM (2014b) Draft genome sequence of uncultivated firmicutes (*Peptococcaceae* SCADC) single cells sorted from methanogenic alkane-degrading cultures. Genome Announc 2:e00909–e00914

Tan B, Semple K, Foght JM (2015) Anaerobic alkane biodegradation by cultures enriched from oil sands tailings ponds involves multiple species capable of fumarate addition. FEMS Microbiol Ecol 91:fiv042

Tang J, Zhuang L, Ma J et al (2016) Secondary mineralization of ferrihydrite affects microbial methanogenesis in *Geobacter-Methanosarcina* cocultures. Appl Environ Microbiol 82:5869–5877

Vedoy DRL, Soares JBP (2015) Water-soluble polymers for oil sands tailing treatment: a Review. Can J Chem Eng 93:888–904

von Netzer F, Pilloni G, Kleindienst S et al (2013) Enhanced gene detection assays for fumarate-adding enzymes allow uncovering of anaerobic hydrocarbon degraders in terrestrial and marine systems. Appl Environ Microbiol 79:543–552

Voordouw G (2012) Interaction of oil sands tailings particles with polymers and microbial cells: first steps toward reclamation to soil. Biopolymers 99:257–262

Wang Y, Zeng W, Qiu G et al (2014) A moderately thermophilic mixed microbial culture for bioleaching of chalcopyrite concentrate at high pulp density. Appl Environ Microbiol 80:741–750

Warren LA, Kendra KE, Brady AL, Slater GF (2016) Sulfur biogeochemistry of an oil sands composite tailings deposit. Front Microbiol 6:1533

Weber KA, Achenbach LA, Coates JD (2006) Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. Nat Rev Microbiol 4:752–764

Widdel F, Knittel K, Galushko A (2010) Anaerobic hydrocarbon-degrading microorganisms: an overview. In: Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 1997–2021

Wilson SL, Li C, Ramos-Padron E et al (2016) Oil sands tailings ponds harbour a small core prokaryotic microbiome and diverse accessory communities. J Biotechnol 235:187–196

Xingyu L, Rongbo S, Bowei C et al (2009) Bacterial community structure change during pyrite bioleaching process: effect of pH and aeration. Hydrometallurgy 95:267–272

Yamada C, Kato S, Ueno Y, Ishii M, Igarashi Y (2014) Inhibitory effects of ferrihydrite on a thermophilic methanogenic community. Microbes Environ 29:227–230

Yao D, Zhanga X, Wanga G et al (2017) A novel parameter for evaluating the influence of iron oxide on the methanogenic process. Biochem Eng J 125:144–150

Yue S, Ramsay BA, Ramsay JA (2015) Biodegradation of naphthenic acid surrogates by axenic cultures. Biodegradation 26:313–325

Zedelius J, Rabus R, Grundmann O et al (2011) Alkane degradation under anoxic conditions by a nitrate-reducing bacterium with possible involvement of the electron acceptor in substrate activation. Environ Microbiol Rep 3:125–135

Zengler K, Richnow HH, Rosselló-Mora R, Michaelis W, Widdel F (1999) Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 401:266–269

Zhang J, Dong H, Liu D et al (2012) Microbial reduction of Fe(III) in illite–smectite minerals by methanogen *Methanosarcina mazei*. Chem Geol 292-293:35–44

Zheng S, Wang B, Liu F, Wang O (2017) Magnetite production and transformation in the methanogenic consortia from coastal riverine sediments. J Microbiol 55:862–870

Zhou S, Xu J, Yang G, Zhuang L (2014) Methanogenesis affected by the co-occurrence of iron (III) oxides and humic substances. FEMS Microbiol Ecol 88:107–120

Zhu R, Liu Q, Xu Z et al (2011) Role of dissolving carbon dioxide in densification of oil sands tailings. Energy Fuel 25:2049–2057

Zhu M-Y, Peng S-C, Tao W et al (2017) Response of methane production and microbial community to the enrichment of soluble microbial products in goethite-dosed anaerobic reactors. Fuel 191:495–499

Microbial Ecology of Naphthenic Acid (NA) Degradation

14

Karl Skeels and Corinne Whitby

Contents

Abstract

Bitumen extraction produces large amounts of oil sands process-affected waters (OSPW) which are stored in vast tailings ponds. OSPW is acutely toxic to many organisms, and this toxicity is largely attributed to the presence of naphthenic acids (NAs). NAs are complex mixtures of organic compounds, including acyclic and cyclic, saturated and aromatic carboxylic acids, which traditionally fit the general formula $C_nH_{2n+z}O_2$. Di-, tri-, tetra-, and pentacyclic diamondoid acids as well as structures that contain more than two oxygen atoms (Oxy-NAs) and/or nitrogen and sulfur have also been identified. NAs may originate from either anthropogenic (e.g., tailings ponds) or natural (e.g., Athabasca River sediments) sources. Although many studies have focused on the biodegradation of either

K. Skeels · C. Whitby (✉)
School of Biological Sciences, University of Essex, Essex, UK
e-mail: ktskee@essex.ac.uk; cwhitby@essex.ac.uk

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_5

model or commercial NAs, many of those NAs found in the environment are recalcitrant to biodegradation. Currently, little is known about the ecology of NA-degrading microorganisms and the range of NAs that they metabolize. Typically, mixed microbial communities from environments that have a history of NA contamination, such as those found in oil sands and OSPW, can degrade NAs, including recalcitrant NAs, more quickly than pure cultures. Indeed, microorganisms capable of effective NA degradation include members of the *Proteobacteria,* particularly *Pseudomonas* spp. However, NA structure and composition, as well as environmental factors such as the presence of specific electron acceptors, trace metals, and competition for substrates from non-NA-degrading microbes, are important drivers in shaping NA-degrading microbial communities. In order to elucidate the mechanisms of NA degradation for future remediation strategies, it is important to better understand the ecology of NA degradation.

List of Abbreviations

AEO	Acid-extractable organics
AS	Activated sludge
BTEX	Benzene, toluene, ethylbenzene and xylene
CHAA	Cyclohexane acetic acid
CHBA	Cyclohexane butyric acid
CHCA	Cyclohexane carboxylic acid
CHPA	Cyclohexane propionic acid
CHPeA	Cyclohexane pentanoic acid
DA	Decanoic acid
DHNA	Decahydro-2-naphthoic acid
GAC	Granular activated carbon
H_2	Hydrogen
HA	Hexanoic acid
LC	Lethal concentration
MCHCA	Methyl-cyclohexane carboxylic acid
MFT	Mature fine tailings
NA	Naphthenic acids
OSPW	Oil sands process-affected waters (or oil sands process waters)
Oxy-NAs	Oxy-naphthenic acids
PAHs	Polycyclic aromatic hydrocarbons
SBR	Sequencing batch reactor
SRB	Sulfate-reducing bacteria

1 Introduction

Petroleum hydrocarbons found in heavy and superheavy oils can be partially oxidized by microorganisms over geological time to form bituminous sands. One of the largest deposits of bituminous sands is in northern Alberta, Canada, which

contains approximately 270 × 10^9 m^3 (1.6 × 10^{12} billion barrels) of bitumen (Chalaturnyk et al. 2002). To extract the bitumen, surface mining operations use the Clark hot water process, which results in the production of vast quantities of tailings (or oil sands process-affected waters (OSPW)). These tailings comprise mixtures of water, sand, fines (clay particles <44 μm), and residual bitumen, which have to be stored in vast tailings ponds (Chalaturnyk et al. 2002; Whitby 2010). In the Fort McMurray region of Alberta alone, around 4.9 million m^3 d^{-1} of tailings have accumulated, and this volume will continue to increase until appropriate remediation technologies are available (Bauer 2015). Of particular concern is the presence of the toxic, acid-extractable organic fractions, traditionally known as naphthenic acids (NAs), which occur naturally in bitumen and accumulate in OSPW (Frank et al. 2008; Whitby 2010).

1.1 What Are Naphthenic Acids (NAs)?

NAs have been classically defined as complex mixtures of organic compounds including acyclic and cyclic, saturated and aromatic carboxylic acids, which traditionally fit the general formula $C_nH_{2n+z}O_2$, where n is the number of carbon atoms and Z is either zero or a negative integer representing the number of hydrogen atoms lost due to ring formation (Fig. 1) (Clemente and Fedorak 2005; Whitby 2010). However, due to the structural complexity of NAs, many naturally occurring NAs are poorly defined (Headley et al. 2016). Advances in high-resolution analytical approaches have also identified di-, tri-, tetra-, and pentacyclic diamondoid acids in OSPW (Lengger et al. 2015; Rowland et al. 2011; Wilde et al. 2015). NA structures have also been identified that contain more than two oxygen atoms and/or other heteroatoms such as nitrogen and sulfur (Clemente and Fedorak 2005; Grewer et al. 2010; West et al. 2014). Indeed, oxy-naphthenic acids (oxy-NAs) (i.e., naphthenic

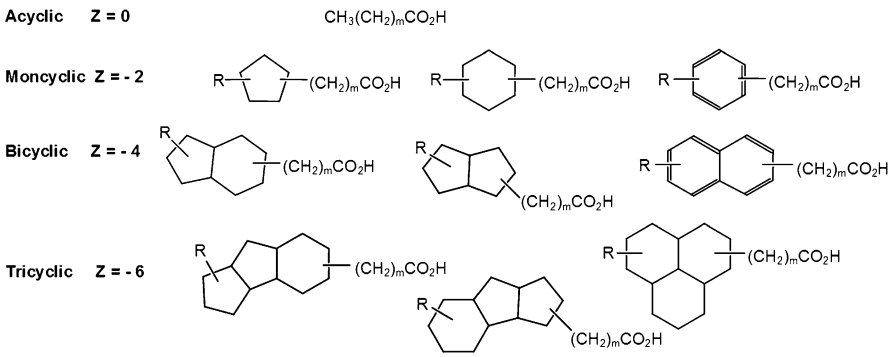

Fig. 1 Examples of NA structures. *R* is a small aliphatic group such as methyl, *Z* is loss of hydrogen atoms due to ring formation, *m* is the number of CH_2 units. (Whitby 2010, modified from Brient et al. 1995)

acid structures with the formula $C_nH_{2n+Z}O_x$, where x = 2 to 5 (Barrow et al. 2009)) are major components of NA mixtures in wastewaters from petroleum industries (Wang et al. 2013).

The chemical and physical properties of NAs are dictated by their structure, and the structural composition of NA mixtures may change over time (Whitby 2010). For example, "aged" OSPW has been shown to contain a larger proportion of high molecular weight NAs (predominantly from the Z = −4, −6 families) compared to "fresh" OSPW which often contains NAs from the Z = 0 family, with palmitic and stearic acids being major components (Grewer et al. 2010; Whitby 2010). High molecular weight C_{80} isoprenoid tetraacids (ARN acids) have also been identified in calcium naphthenate deposits (Baugh et al. 2004; Lutnaes et al. 2007; Magnusson et al. 2006, 2008; Morii et al. 1998; Smith et al. 2007). NA concentration may also vary depending, in part, on geological and hydrological factors, as well as aerial deposition from oil-upgrading activities (Kelly et al. 2009, 2010; Kurek et al. 2013) and microbial biodegradation rates (Whitby 2010). For example, NA concentrations in "fresh" OSPW may be as high as 120 mg L^{-1}, but even after many decades of microbial *in situ* degradation, NA concentrations may remain around 19 mg L^{-1} in some tailings ponds (Holowenko et al. 2002). NA degradation is also influenced by both carbon number and the spatial arrangement of the alkyl side branch (Johnson et al. 2011, 2013; Misiti et al. 2014; Smith et al. 2008).

NAs demonstrate both acute and chronic toxicity to several organisms including microorganisms (Whitby 2010). The degree of toxicity is generally related to molecular weight, with higher molecular weight acids often demonstrating greater acute toxicity (Holowenko et al. 2002). Aromatic NAs, which comprise >30% of the NAs in OSPW relative to the acyclic acids (Jones et al. 2012), contribute disproportionally to the overall toxicity of NAs (Headley et al. 2004; Johnson et al. 2011). For example, while alicyclic acids are toxic (LC_{50} 13.1 mg L^{-1}), the higher molecular weight aromatic acids are somewhat more toxic, at least on a weight per volume basis (LC_{50} 8.1 mg L^{-1}) (Scarlett et al. 2013). Acute toxicity of NAs may also be associated with the surfactant properties of NAs, changing membrane properties (Schramm et al. 2000; Frank et al. 2009).

Since NAs contribute toward the toxicity of tailings pond waters, studies on NA biodegradation have been extensive. Despite this, little is known about the ecology of NA-degrading microorganisms and the range of NAs that they metabolize. Previously, our understanding of the microorganisms driving NA biodegradation was largely through enriching consortia on individual NAs and/or NA mixtures (Clemente et al. 2004) or by isolating pure cultures (Whitby 2010). However, with the advent of high-throughput sequencing platforms, the ecology of NA-degrading microorganisms found in different ecosystems is now emerging. This chapter aims to review the ecology of microorganisms found in NA-contaminated environments, many of which are presumed to be involved in NA degradation, with some proven to degrade NAs. This chapter also aims to unravel the complex microbial communities found across different NA-contaminated ecosystems.

2 Ecology of Microorganism-Degrading Model and Commercial Naphthenic Acids

The source of NAs, their structural composition, concentration, toxicity, and chemistry may differ, which in turn may influence the ecology of the *in situ* NA-degrading microbial communities. Many early studies on NA degradation utilized either model (surrogate) NAs (i.e., NAs chemically synthesized in the laboratory) or commercial NA mixtures (i.e., NA fractions obtained during petroleum processing, which were available commercially). Both model and commercial NAs are generally more readily degraded than those NAs found in the environment (Clemente et al. 2004; Clemente and Fedorak 2005; Del Rio et al. 2006; Johnson et al. 2011, 2012, 2013; Rho and Evans 1975; Scott et al. 2005). Previous studies, using a range of different model NAs,have identified several NA-degrading microorganisms, including *Mycobacterium* sp., *Brevibacterium* sp., *Achromobacter* sp., *Corynebacterium* sp., *Rhodococcus* sp., *Acinetobacter* sp., *Alcaligenes* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Moraxella* spp., *Micrococcus* sp., and *Bacillus* sp. (reviewed in Whitby 2010). Model NAs have also been important in highlighting how NA structure relates to differences in biodegradation rates and levels of NA persistence. For example, differences in NA degradation rates have been found with different geometric isomers of NAs (Headley et al. 2002b). Specifically, heterotrophic microbial populations from Athabasca River samples degraded the *trans*-isomers of model NAs more rapidly than the respective *cis*-isomers (Headley et al. 2002b). In another study, the degree of alkyl branching of four aromatic alkanoic acid isomers affected NA biodegradation rates, and 16S rRNA gene sequences relating to *Pseudomonas* spp., *Burkholderia* spp., and *Sphingomonas* spp. were found to be dominant during biodegradation (Johnson et al. 2011). *Ochrobactrum* spp., *Brevundimonas* spp., and *Bacillus* spp. have also been shown to have higher metabolic activity on polycyclic aromatic NAs compared to other classes of NA surrogates (Yue et al. 2015). Several different microorganisms, including *Pseudomonas citronellolis* and *Mycobacterium austroafricanum*, have also demonstrated specialized metabolic capabilities to degrade carboxylic acids with alkyl-substituted aliphatic chains (Fall et al. 1979; Marchal et al. 2003; Smith et al. 2008). However, one study using a commercial NA mixture (NA sodium salt) revealed that, although significant degradation (~85%) was possible aerobically, only a small fraction of the NA mixture was completely mineralized to CO_2 (Misiti et al. 2013). Analysis of the 16S rRNA gene clone library showed that 80% of the sequences belonged to the *Gammaproteobacteria* and the community was largely dominated by known NA degraders, such as *Pseudomonas putida* and *Pseudomonas fluorescens* as well as known hydrocarbon-degrading bacteria found in OSPW, such as *Microbulbifer* and *Xanthomonas* (Misiti et al. 2013). *Methylophilus* and *Methylobacillus*, which are known methanol-utilizing bacteria, also accounted for a large proportion (63–64%) of the community, although the role these microbes play *in situ* is not fully understood (Misiti et al. 2013).

The type and availability of electron acceptors are also important in shaping the microbial NA-degrading community. One study that used simple model NAs, such as cyclohexane carboxylic acid (CHCA), cyclohexane acetic acid (CHAA),

cyclohexane propionic acid (CHPA), cyclohexane butyric acid (CHBA), and cyclohexane pentanoic acid (CHPeA), showed they were readily biodegraded by different microbial taxa, depending on the anaerobic electron-accepting conditions (Clothier and Gieg 2016). Specifically, cultures amended with CHCA under sulfate-reducing conditions resulted in a dominance of *Desulfobulbus* and *Desulfomicrobium* sequences, while under nitrate-reducing conditions, sequences relating to *Pseudomonas*, *Terrabacter*, *Limnobacter, Lutibacter*, and *Derxia* were abundant (Clothier and Gieg 2016). Methanogenic enrichments were dominated by sequences relating to *Clostridium* and *Methanosaeta* (Clothier and Gieg 2016). Interestingly, under iron-reducing conditions, cultures were primarily composed of methanogenic Archaea such as *Methanosarcina* and *Methanoculleus*. The authors postulated that the methanogens were using H_2 to produce CH_4 and during this process transferred some electrons to Fe(III), reducing it to Fe(II) (Clothier and Gieg 2016). This link between iron reduction and methanogenesis has also been suggested previously (Siddique et al. 2014). The most abundant bacterium under iron-reducing conditions was *Trichococcus*, a known citrate-fermenting microorganism (Clothier and Gieg 2016). The authors suggest that the microbial communities in the NA-amended Fe (III)-reducing cultures most likely shifted over time from initially biodegrading NAs to utilizing citrate (Clothier and Gieg 2016). Another study which enriched a microbial community derived from an oil sands tailings pond under either anaerobic sulfate-reducing or methanogenic conditions found no significant degradation of a diamondoid NA (adamantane-1-carboxylic acid) or acid-extractable NAs from OSPW, despite incubation conditions being amenable for the biodegradation of the PAH, 2-methylnaphthalene (Folwell et al. 2015). This suggests that NAs, particularly high molecular weight NAs, could persist in the anoxic layers of OSPW (Folwell et al. 2015).

3 Ecology of the Microorganisms Found in NA-Contaminated Environments

It is important that we better understand the ecology of microbial communities found in different NA-contaminated environments. Environmental NAs may originate from both anthropogenic (e.g., tailings ponds) and natural (e.g., Athabasca River) sources. Indeed, NA concentrations between 0.1 and 0.9 mg L^{-1} have been found in the Athabasca River (Schramm et al. 2000) and in aquifers at >55 mg L^{-1} (CONRAD 1998). During the biodegradation of crude oil, the linear and lower molecular weight NAs (<22 carbons) are biodegraded preferentially. Consequently, there is an increase in the relative concentration of specific hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs) (such as alkylnaphthalenes or alkylphenanthrenes), NAs, resins, and asphaltenes which resist further degradation (Clemente and Fedorak 2005; Shuqing et al. 2008). The NAs found in various environments, including OSPW, tailings, and Athabasca River sediments, are often more recalcitrant to biodegradation (Scott et al. 2005). Often the microorganisms that degrade commercial or model NAs are unable to metabolize environmental NAs

(Bataineh et al. 2006; Headley et al. 2010). Generally, the most recalcitrant NAs to biodegradation found in the environment have structures consisting of more highly branched alkyl chains (Han et al. 2008; Smith et al. 2008) or diamondoid structures (Folwell et al. 2015; Rowland et al. 2011). Thus, indigenous NA-degrading microbial populations must be able to thrive in the complex and toxic environments that are associated with NAs (Brient et al. 1995).

3.1 River Sediments

Sediments from the Athabasca River and its tributaries naturally contain bitumen at different concentrations, yet little is known about the impact of this variation on the ecology of the microbial river communities (Wong et al. 2015; Yergeau et al. 2013). In addition, the microbial communities in the Athabasca watershed are continuously exposed to varying bituminous components through erosion of the oil sands by the river flow and aerial deposition from oil-upgrading activities (Kelly et al. 2009, 2010; Kurek et al. 2013). Parts of the Athabasca River near the oil sands mining operations are also considered to be nitrogen and phosphorus limited, and so the in situ microbial communities may also be under nutrient stress (Chambers et al. 2006). Indeed, microbial NA degradation rates can be inhibited by phosphorus and/or nitrogen limitations (Herman et al. 1994; Lai et al. 1996).

For some time, it has been known that microorganisms of the Athabasca watershed have the potential to degrade NAs (Del Rio et al. 2006), suggesting that the *in situ* microbial communities can potentially bioremediate oil sands tailings and OSPW. Specifically, river sediment communities that degraded the hydrocarbon components in bitumen were largely dominated by members of the *Proteobacteria* (such as *Pseudomonas* sp., *Xanthomonas* sp.) (Yergeau et al. 2012; Del Rio et al. 2006; Wyndham and Costerton 1981a, b). In a study by Yergeau et al. (2012), the microbial communities in the fine tailings of two tailings ponds were compared to the sediments from sites in the Athabasca River and tributary (Firebag Creek, Steepbank Creek, and Ells River). Based on amplicon sequencing, the microbial communities in the fine tailings were distinct from those found in the Athabasca River and its tributary sediments (Yergeau et al. 2012). For all samples, *Proteobacteria* was the dominant phylum, with *Alphaproteobacteria* and *Betaproteobacteria* being the most dominant classes, with the exception of Steepbank Creek and upper Athabasca tributary where *Deltaproteobacteria* dominated (Yergeau et al. 2012). *Bacteroidetes*, *Firmicutes*, and *Chloroflexi* were also found in relatively high abundance (Yergeau et al. 2012).

The bacterial and archaeal communities found in the river sediments located in close proximity to tailings ponds were more similar to each other (and to those from the tailings ponds) compared to communities further away from the tailings ponds, suggesting that proximity to tailings is important in shaping the microbial communities in these river sediments (Yergeau et al. 2012). Although there is no deliberate discharge of contaminated tailings ponds into the environment, there may be leaching or seepage of tailings water into neighboring aquatic ecosystems (Yergeau

et al. 2012). Not surprisingly the bacterial diversity has generally been found to be lower in sediments from Steepbank Creek which is more heavily disturbed by mining operations than Ells River and Firebag Creek (Yergeau et al. 2012). *Mycobacterium* spp., *Nocardia* sp., and the yeast *Rhodotorula* sp., which degrade the components of bitumen, have also been identified from the Athabasca River and its surrounding tributaries (Wyndham and Costerton 1981a, b). In another study, river and creek bed samples from along the Horse River and Saline Creek in the Fort McMurray region of Alberta, Canada, showed an abundance of *Chitinophagaceae*, *Rhizobiales*, *Rhodocyclaceae*, *Comamonadaceae*, and *Sphingomonadaceae* sequences (Wong et al. 2015).

3.2 Tailings Ponds

Tailings ponds are typically stratified, and oxygen ingress is generally limited to the upper surface layers, below which they become anoxic (~1 m below the surface and deeper) (Ramos-Padron et al. 2011; Stasik and Wendt-Pethoff 2014). Yet, tailings ponds harbor diverse microbial taxa that influence the properties and biogeochemistry of the tailings through various redox reactions (An et al. 2013; Foght et al. 2017; Foght 2015; Penner and Foght 2010; Stasik et al. 2014; Siddique et al. 2018). Many of the microorganisms found in tailings are involved in anaerobic hydrocarbon degradation and include fermentative and syntrophic bacteria, nitrate reducers, iron reducers, sulfate-reducing bacteria (SRB), and H_2- and acetate-using methanogens (An et al. 2013; Golby et al. 2012; Ramos-Padron et al. 2011; Siddique et al. 2007, 2011). Methanogenic activity is commonly observed in tailings ponds typically as a result of the biodegradation of residual bitumen and naphtha (Fedorak et al. 2003; Holowenko et al. 2000). Indeed, methane production has been shown to reach up to 43,000 m^3/day in some ponds (Siddique et al. 2011). However, the addition of sulfate from gypsum (calcium sulfate dihydrate) as a tailings processing aid can inhibit methanogenesis, resulting in sulfidogenic activity (Fedorak et al. 2003; Ramos-Padron et al. 2011). Furthermore, SRB have been found in oil sands fine tailings wastes that may outcompete methanogens for available substrates when sulfate is abundant (Foght et al. 1985; Holowenko et al. 2000). For example, one study showed that the aerobic surface layer, which had the highest concentration of sulfate (6 mM) but no sulfide, had a very different microbial community compared to the rest of the pond (Ramos-Padron et al. 2011). The lack of sulfide in the aerobic surface layers was considered to be due to either its chemical or microbial oxidation (Ramos-Padron et al. 2011). Laboratory incubations using anaerobic tailings collected from a depth where methanogenesis was measured as relatively high showed that increasing sulfate concentration inhibited methane production (Ramos-Padron et al. 2011). In the deeper anaerobic zones (where sulfide and sulfate concentrations increased), the microbial community was dominated by sequences relating to syntrophs (*Pelotomaculum*, *Syntrophus*, and *Smithella* spp.) and sulfate- and sulfur-reducing bacteria (*Desulfocapsa*, *Desulfurivibrio* spp.). Other anaerobes associated with hydrocarbon utilization or iron and sulfur cycling, including the SRB

(*Desulfocapsa*, *Desulfurivibrio*, *Desulfobacterium*, and *Desulfuromonas*), were also recovered (Ramos-Padron et al. 2011). Other genera were only abundant in the non-surface samples, e.g., *Leptolinea*, *Thauera*, *Rhodoferax*, and *Acidovorax* (Ramos-Padron et al. 2011). The deepest zones of the tailings pond harbored bacteria not found elsewhere, e.g., *Brachymonas*, *Thiobacillus*, and *Cellulomonas,* and it is postulated that these microorganisms may contribute to tailings densification (i.e., consolidation) (Ramos-Padron et al. 2011), as has been suggested previously for *Thauera* spp. using a laboratory study (Bordenave et al. 2010).

Bacterial sequence diversity can often be lower in tailings ponds compared to other NA-contaminated ecosystems such as the Athabasca River sediments, which is partly due to a greater concentration of toxic NAs as well as limited carbon sources (Yergeau et al. 2012). This reduced bacterial diversity may have implications on the rate at which reclamation operations can occur (Yergeau et al. 2012). Indeed, it has been suggested that a diverse microbial community is crucial for effective tailings bioremediation (Headley and McMartin 2004).

In tailings, members of the *Beta-, Delta-, and Epsilonproteobacteria* have been found (An et al. 2013; Siddique et al. 2011; Yergeau et al. 2012). Specifically, within the *Beta- and Deltaproteobacteria*. *Rhodoferax*, *Thiobacillus*, and *Smithella* were dominant, and the following six genera had a positive correlation with NA degradation: *Schumannella* (*Actinobacteria*), *Hydrogenophaga* (*Betaproteobacteria*), *Azonexus* (*Betaproteobacteria*), *Salinimicrobium* (*Bacteroidetes*), *Achromobacter* (*Betaproteobacteria*), and *Gillisia* (*Bacteroidetes*) (Yergeau et al. 2012). *Alcaligenes* spp. and *Acinetobacter spp.* have also been found in tailings ponds depending on the depth the samples were obtained from (Foght et al. 1985). *Pseudomonas*, *Thauera*, *Rhodoferax*, *Acidovorax*, *Thiobacillus Actinobacteria*, *Kurthia*, and *Brachymonas*, among others, have also been recovered from tailings ponds (Del Rio et al. 2006; Herman et al. 1994; Holowenko et al. 2000; Ramos-Padron et al. 2011; Siddique et al. 2006; Wyndham and Costerton 1981a, b).

Within the Archaea, members of *Methanomicrobiales*, *Methanobrevibacter, Methanosaeta*, and *Methanobacteriales* have been found to be dominant contributors to methanogenesis during hydrocarbon and/or NA degradation in tailings ponds (Abu Laban et al. 2015; An et al. 2013; Siddique et al. 2011; Yergeau et al. 2012). Yergeau et al. (2012) showed that archaeal sequence libraries obtained from tailings ponds were composed mainly of *Euryarchaeota* (primarily *Methanomicrobia*), with the *Crenarchaeota* almost absent, while in the deep anaerobic zones (where sulfide and sulfate concentrations increased), methanogens (e.g., *Methanosaeta* (acetate-using) and *Methanolinea* and *Methanoregula* (H_2-using)) were present (Ramos-Padron et al. 2011).

Studies using laboratory enrichment cultures derived from tailings have also revealed a dynamic selection process of certain bacteria depending on the substrate and conditions (Aubu Laban et al. 2014, 2015; Tan et al. 2013). For example, when aromatics were present, *Desulfosporosinus* (under methanogenic conditions) or Desulfobulbaceae (under sulfidogenic conditions) dominated. In contrast, when alkanes were present, other species such as *Peptococcaceae* dominated (Aubu Laban et al. 2014, 2015; Tan et al. 2013). The addition of organic compounds may

also enhance NA degradation by some microorganisms by acting as co-metabolites (Dutta and Harayama 2001). However, the added compounds or their metabolites may be preferentially degraded by the *in situ* communities and also may be toxic to other indigenous NA-degrading microbes. Other readily utilized carbon sources, such as glucose, may also be important additives for maintaining the population size of NA degraders (Allard and Neilson 1997; Demeter et al. 2015a).

Ozonation, which has been shown to be a potential treatment strategy for OSPW, can also shape the microbial ecology of tailings ponds. One study showed that ozonation tends to decrease the microbial diversity of planktonic microbes but increase diversity in biofilms (Islam et al. 2014b). Specifically, ozonation decreased proteobacterial sequence abundance compared to non-ozonated biofilm samples (Islam et al. 2014b). Although *Gammaproteobacteria,* particularly *Pseudomonadales,* were dominant in OSPW, when the OSPW was treated by ozonation, drastic changes were found to occur in the microbial community; primarily there was an increase in dominance of *Burkholderiales,* while members of the *Pseudomonadales,* which were sensitive to ozonation, decreased (Islam et al. 2014b).

Generally, the prokaryotic communities within tailings ponds have been shown to consist of a few core taxa and numerous accessory members that likely afford resilience and functional redundancy including roles in iron, nitrogen, and sulfur cycling, syntrophy, fermentation, and methanogenesis (Wilson et al. 2016). This flexibility and adaptation of the microbial community is an important consideration for pond reclamation when certain substrates are being selectively removed.

3.3 Bitumen-Saturated Sandstone Outcrops

Oil sand deposits can also be found naturally exposed in surface outcrop sections, such as those along the Athabasca-Clearwater River drainage networks, where they are further biodegraded by both aerobic and anaerobic processes (Wong et al. 2015). The indigenous microbial communities in outcrops are part of a unique ecosystem and may be important in NA biodegradation, yet they are poorly understood. A study of bitumen-saturated sandstone retrieved from outcrop cliffs in northeastern Alberta identified a network of taxa-spanning domains and included fungi and hydrocarbon-, methane-, or acetate-oxidizing heterotrophic bacteria, distinct from the heterotrophic community found in nearby river sediments (Wong et al. 2015). Specifically, outcrop cliff communities were dominated by *Acetobacteriaceae*, *Beijerinckiaceae*, *Rhizobiales*, *Methylobacterium*, *Methanoculleus*, *Clostridia*, *Methanosaeta*, *Mycobacterium*, and *Acidobacterium* sequences, and among the fungi, *Protomyces* and *Coniosporium* sequences were abundant (Wong et al. 2015). Anaerobes, such as methanogens (*Methanomicrobiales*, *Methanosarcinales*, and *Methanobacteriales*), *Clostridiales*, and syntrophs (*Syntrophobacterales* and *Anaerolineales*), were also identified (Wong et al. 2015).

Interestingly, a large proportion of the outcrop library comprised of thermophilic microorganisms, including *Methanothermobacter*, *Thermotaga*, *Thermomonas*,

Caldanaerobacter, *Caldimicrobium*, and *Thermanaeromonas,* and was likely due to the high ambient summer temperatures experienced on the outcrop slopes between 55 °C and 60 °C (Wong et al. 2015). Co-occurrence analyses of the microbial communities identified two distinct networks in the cliff tablets with Network I comprising largely of aerobic putative hydrocarbon degraders, such as *Burkholderiales*, *Flavobacteriales*, *Pseudomonadales*, *Sphingomonadales*, anaerobic methanogens (*Methanomicrobiales*, *Methanosarcinales*, and *Methanobacteriales*), *Clostridiales*, and syntrophs (*Syntrophobacterales* and *Anaerolineales*), and Network II comprising aerobic bacteria and fungi including *Dothideales*, *Erythrobasidiales*, *Microthamniales*, *Russulales*, *Sporadotrichida*, *Sporidiobolales*, and *Taphrinales* as well as the putative hydrocarbon-, C_{2}-, or C_{1}-oxidizing bacteria *Rhizobiales* (*Beijerinckiaceae* and *Methylobacterium*) and Rhodospirillales (*Acetobacteraceae*) (Wong et al. 2015). These findings suggest that hydrocarbon degradation in this environment may be initiated by fungi, yielding products which are metabolized predominantly by the aerobic bacterial community and the anaerobic component of Network I playing a minor role (Wong et al. 2015).

3.4 NA-Contaminated Wetlands

Wetlands are currently being tested by the oil sands industry to treat OSPW, and companies mining oil sands in Alberta, Canada, face the challenge of reclaiming wetlands under water-use restrictions (Mollard et al. 2015). Wetland reclamation after mining will generate marshes characterized by elevated salinity and residual hydrocarbons (Mollard et al. 2015). Specifically, the microbial communities found in many of the wetlands close to the Athabasca oil sand deposits have a history of long-term exposure to bitumen and the associated NAs therein. Thus, native wetlands that receive OSPW (i.e., NA exposed) are expected to have distinct microbial communities from those wetlands that have not been exposed (Hadwin et al. 2006). One study using denaturing gradient gel electrophoresis showed that bacterial communities obtained from exposed wetlands were more homogeneous compared to those found in unexposed wetlands (Hadwin et al. 2006). The time required to alter the community is currently unknown, yet the microbial communities found in exposed wetlands have been shown to degrade the NAs in OSPW faster than microbial communities from non-exposed wetlands (Del Rio et al. 2006; Hadwin et al. 2006; Headley et al. 2000). Currently, little is known about the microbial ecology in NA-contaminated wetlands and a better understanding of these communities in relation to NA degradation would be useful in removing NAs in OSPW and other sites impacted by bitumen.

One study used eleven wetlands, eight of which had prior exposure to varying degrees of OSPW and NA-contaminated materials by dyke seepage or direct exposure (Del Rio et al. 2006). The ability of the microbial communities in the various wetlands to degrade the model compounds cyclohexane carboxylic acid (CHCA) and decahydro-2-naphthoic acid (DHNA) was assessed. Results showed that mineralization rates for CHCA were relatively uniform across all sites, suggesting that

microbial communities in wetlands with no previous exposure to NAs were capable of degrading monocyclic NAs (i.e., those belonging to the $Z = -2$ family). A different pattern was observed with DHNA, whereby the extent on degradation by the non-exposed sites was significantly lower compared to NA-exposed wetlands, suggesting that once wetlands are exposed to even low levels of OSPW (i.e., low-impact sites), the microbial communities have adapted to degrade the bicyclic NAs (i.e., those belonging to the $Z = -4$ family) (Del Rio et al. 2006). In these wetlands, *Pseudomonas putida* and *Pseudomonas fluorescens* were isolated from NA-degrading enrichment cultures (Del Rio et al. 2006).

Although the microbial communities in wetlands can reduce the total NAs in OSPW, it has been shown that they can require long residence times (i.e., 400 days) before significant NA reduction is observed (Toor et al. 2013). Furthermore, the more persistent components of NA mixtures that appeared to be associated with residual chronic toxicity can often remain (Toor et al. 2013). Additionally, in wetland environments, nitrification may inhibit the mineralization of NAs as NA-degrading communities may be outcompeted by nitrifiers for available oxygen and nutrients, such as nitrogen and phosphorus (Lai et al. 1996). Thus, wetlands are largely ineffective in eliminating toxicity as many of the NAs present are recalcitrant to natural biodegradation. Despite this, it may be advantageous to "seed" any new wetlands intended for remediation with known NA-degrading sediment communities, and further research into this area is required.

3.5 Biofilms and Bioreactors

It has been shown previously that the close proximity of cells growing as a biofilm can facilitate substrate degradation, whether this is through genetic or metabolite exchange, concentration of microbial biomass, or conferring resistance to various stressors (Chakraborty et al. 2012; Nicolella et al. 2000; Singh et al. 2006). It has also been shown that differences in NA-degrading microbial communities may also occur between planktonic versus biofilm cultures, which in turn influences NA degradation rates (Demeter et al. 2015a; Folwell et al. 2016). For example, one study showed that the addition of high molecular weight PAHs led to a shift in the bacterial and fungal community composition, but thereafter the major factor determining the fungal community composition was whether they were in the planktonic phase or attached to filters, whereas, the major determinant of the bacterial community composition was the nature of the PAH serving as the carbon source (Folwell et al. 2016). In another study, planktonic cultures of *Cyanobacteria* were found to degrade hexanoic acid (HA), decanoic acid (DA), and cyclohexane butyric acid (CHBA), yet when grown as a biofilm, the *Cyanobacteria* failed to degrade any of the NAs tested (Demeter et al. 2015a). However, sequences relating to *Cyanobacteria*, *Bacteroidetes*, and *Firmicutes* decreased in abundance when exposed to high concentrations of bituminous compounds (Yergeau et al. 2013). Indeed, *Cyanobacteria* could be used as a bioindicator to monitor the impact of oil sands mining operations (Yergeau et al. 2013). In another study, *Emiliania huxleyi*

(*a* marine coccolithophore) has been shown to be highly sensitive to specific model NAs and may also have the potential to be used as a bioindicator to track specific NA pollutants in marine environments (Beddow et al. 2016). However, of particular concern, is the lowered photosynthetic activity observed in biofilms exposed to NA-contaminated sediments (Yergeau et al. 2013).

Often mixed microbial consortia are required for the complete biodegradation of the more recalcitrant model NAs, such as those compounds with methyl substitutions on the cycloalkane rings (Demeter et al. 2015a; Headley et al. 2002a, b; Herman et al. 1993; Smith et al. 2008). Demeter et al. (2015a) reported that although *Rhodococcus* spp. were found to degrade the greatest number of model NAs tested, this was still less than when mixed species cultures were added. Mixed biofilms may therefore be useful for removing the NAs in OSPW (McKenzie et al. 2014). One potential approach to remove and detoxify NAs from OSPW is to exploit mixed NA-degrading biofilm communities in bioreactors (either as batch or as continuous biofilm) (Choi et al. 2014; Golby et al. 2012; Islam et al. 2014a; Paslawski et al. 2009). In order to improve the design and efficiency of biofilm reactors as a treatment process, a better understanding of the microbial community composition is required. Choi et al. (2014) used two sequencing batch reactors (SBR) inoculated with either activated sludge (AS) or mature fine tailings (MFT) to treat the NAs in OSPW. Maximum removal rates of acid-extractable organics (AEO) were 8.7% and 16.6% in the AS-SBR and the MFT-SBR, respectively. Pyrosequencing analysis revealed that *Proteobacteria* particularly members of the *Beta-* and *Gammaproteobacteria* were dominant in both reactors. *Proteobacteria* and specifically *Alpha-* and *Betaproteobacteria* have also been found to be abundant in rotating bioreactors as identified through ion torrent 16S rRNA gene sequencing (Yergeau et al. 2013). In another study, Huang et al. (2012) used a circulating packed bed bioreactor to investigate the biodegradation of trans-4-methyl-cyclohexane carboxylic acid (trans-4MCHCA), a mixture of *cis-* and *trans*-4-methyl-cyclohexane acetic acid (4-MCHAA), and mixture of these three naphthenic acids. Results showed that the maximum biodegradation rate of *trans*-4MCHCA observed during the continuous operation was significantly higher than those reported for continuous stirred tank reactors (Huang et al. 2012). Anoxic biofilm reactors have also shown that NA removal rates were at least twofold higher than the values reported for the aerobic biofilm reactor (Gunawan et al. 2014). Another study by McKenzie et al. (2014) used two immobilized soil/sediment bioreactors (ISBRs) operating in series to treat NAs in OSPW. Analysis of the biofilm community showed a dominance of ammonium- and nitrite-oxidizing bacteria suggesting that nitrification was occurring. The dominant genera included *Nitrosomonas, Candidatus* Nitrotoga*, Arenimonas, Mesorhizobium, Bradyrhizobium, Nitrosospir*a, *Mycobacterium, Limnobacter*, and *Commamonas,* with *Truepera*, *Flexibacter*, and *Saprospiraceae* considered to be involved in the degradation of the more recalcitrant NAs (McKenzie et al. 2014).

One technology is the Calgary Biofilm Device (CBD), which has been designed as a high-throughput method of growing biofilms (Demeter et al. 2015b; Golby et al. 2012). Using this method, between 70% and 80% of the microbial community from the original tailings sample could be maintained under aerobic, microaerobic,

and anaerobic conditions (Golby et al. 2012). 16S rRNA gene pyrotag sequencing showed that the biofilms were dominated by *Proteobacteria* (particularly *Alphaproteobacteria*) (Golby et al. 2012). The most abundant genus in the aerobic biofilms was *Rhodoferax,* followed by *Acidovorax, Acinetobacter, Pseudomonas, and Thioalkalispira*, while in the anaerobic biofilms, *Hydrogenophaga* was the most abundant genus followed by *Rhodoferax*, *Methyloversatilis*, *Magnetospirillum*, and *Acidovorax* (Golby et al. 2012). Archaea, which represented 7% of the original tailings community, were not found in the biofilms (Golby et al. 2012). Another study, using the model organism *Pseudomonas fluorescens,* showed that two biochar samples from softwood bark and Aspen wood facilitated the most microbial biofilm growth and that NA removal increased from 30% to 87% as a result of increased immobilization of Fe, Al, and As on the biofilm-associated biochar (Frankel et al. 2016). Such devices and approaches may facilitate a rapid method for obtaining functional inoculants for NA degradation (Lemire et al. 2015).

Granular activated carbon (GAC) biofilm technology has been shown to remove >86% and 99.5% of NAs from raw and ozonated OSPW, respectively, following GAC treatment (Islam et al. 2014b). The potential for GAC to remove recalcitrant and toxic NAs is largely due to its high adsorptive capacity for organics and the high biomass concentrations found in the established biofilms (Combarros et al. 2014; Frascari et al. 2014). Sequencing was used to characterize the GAC biofilm community and showed that *Proteobacteria* were dominant in GAC biofilms compared to planktonic samples (Islam et al. 2015). In particular, *Alphaproteobacteria* (e.g., *Rhizobiales*, *Rhodospirillales*, and *Rhodobacterales*) and *Gammaproteobacteria* (e.g., *Pseudomonadales*, *Alteromonadales*, *Chromatiales*, *Xanthomonadales*, *Oceanospirillales*, *Legionellales*, and *Methylococcales*) were the most abundant in the GAC biofilms (Islam et al. 2015). This increase in abundance of sequences relating to *Proteobacteria* in the biofilms may be due to the broad degradation ability of this phylum, and indeed, many putative PAH degraders were recovered from the GAC biofilm communities including *Burkholderiales*, *Pseudomonadales*, and *Sphingomonadales* (Islam et al. 2015). Both community richness and diversity were found to decrease in GAC biofilm samples compared to OSPW, which may be due to an accumulation of toxic NAs on the GAC surface allowing only those members of the community with high NA tolerances to survive (Islam et al. 2015). Members of the *Proteobacteria* have also been shown to be positively correlated with NA concentration in aerobic biofilms elsewhere (Yergeau et al. 2013). Many other known PAH degraders, such as *Burkholderiales a*nd *Sphingomonadales,* were also found in the GAC biofilm (Islam et al. 2015).

In contrast to *Alpha-* and *Gammaproteobacteria*, the *Deltaproteobacteria* were not able to grow on the GAC biofilm, which may be due to an increased sensitivity to higher concentrations of NAs found on the GAC surface (Islam et al. 2015). Interestingly, *Nitrospirae*, which are potentially involved in denitrification, sulfur oxidation, and sulfate reduction, were also identified and may have been metabolizing NA compounds containing nitrogen and sulfur (Islam et al. 2015). In addition, *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes*, and *Chloroflexi* were also recovered, and it is possible that members of these bacteria were involved in prolonging operational lifespan of the GAC surfaces (Islam et al. 2015). Previously it has been

reported that the adsorption capacity of GAC may decrease with bioreactor operating time as by-products and extracellular polymeric substances accumulate on the GAC surface (Aktas and Çeçan 2007). In another study, a modified Ludzack-Ettinger membrane bioreactor (MLE-MBR) was continuously operated for 425 days and evaluated for its feasibility for OSPW treatment (Xue et al. 2016). NA removal of 24.7% was observed in the reactor after 361 days of operation. Ultra-performance liquid chromatography/high-resolution mass spectrometry analysis revealed that the removal of individual NA species declined with increased ring numbers. Pyrosequencing analysis showed that *Betaproteobacteria* were dominant in sludge samples from the MLE-MBR, with microorganisms such as *Rhodocyclales* and *Sphingobacteriales* capable of degrading hydrocarbons. Such proposed bioreactor technologies demonstrate good potential for removing recalcitrant organics in OSPW (Islam et al. 2014a, 2015; Xue et al. 2016).

4 Summary

Previous studies, using model and/or commercial NAs, have identified several NA-degrading microorganisms. However, it is often the case that the microbes that can degrade commercial or model NAs are unable to metabolize environmental NAs (Bataineh et al. 2006; Headley et al. 2010). In order to better understand NA degradation, it is important to elucidate the microbial ecology of NA degraders across different environments. Specifically, *Pseudomonas* spp. appear to be ubiquitous and have been identified in a range of NA-exposed ecosystems including Athabasca River sediments, biofilms, bioreactors, outcrops, and tailings ponds (Fig. 2). In contrast to *Pseudomonas* spp., other bacterial species have only been found in certain environments (Fig. 2). Additionally, some methanogenic Archaea, e.g., *Methanobrevibacter*, *Methanolinea*, and *Methanoregula,* have only been identified in tailings ponds (Abu Laban et al. 2015; An et al. 2013; Ramos-Padron et al. 2011; Siddique et al. 2011; Yergeau et al. 2012), while others (*Methanoculleus*, *Methanosarcinales*, and *Methanothermobacter*) were only found in outcrops (Wong et al. 2016) (Fig. 2). Thus, prior exposure to NAs may not necessarily select for similar microbial communities, and other factors are driving the ecology of NA degraders.

5 Research Needs

NAs are an important group of organic pollutants originating from both anthropogenic and natural processes. Although many studies have focused on the biodegradation of either model or commercial NAs, the real challenge lies in removing those NAs found in the environment. Thus, it is important to have a better understanding of the ecology of NA-degrading microorganisms that can metabolize the different compositions of NAs found in the environment. Significant developments in analytical and molecular methods, including high-throughput sequencing and “omics” approaches, have made some headway

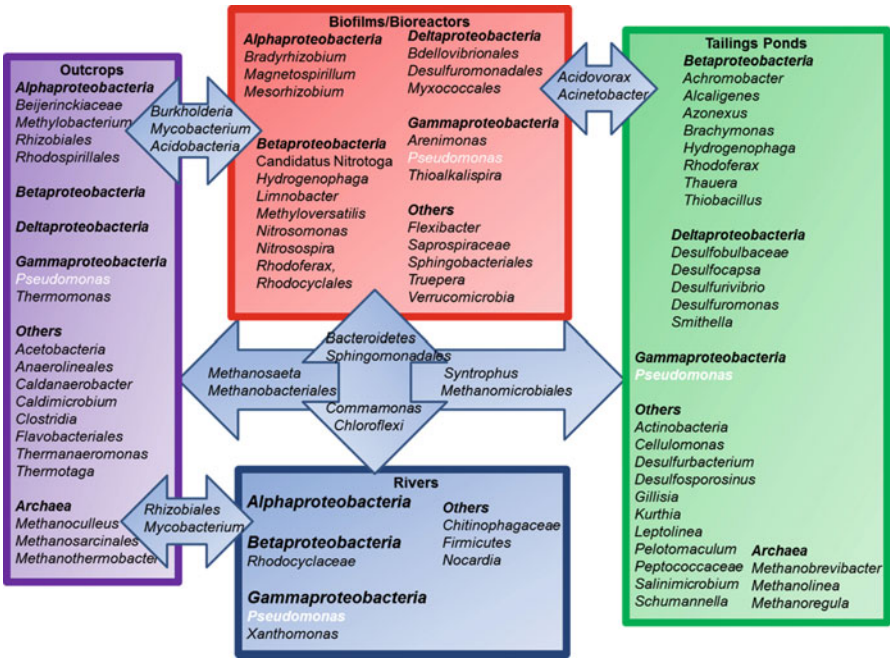

Fig. 2 Conceptual diagram summarizing the main microorganisms identified from different NA-contaminated environments. Taxa indicated in double arrows are found in both environments. *Pseudomonas* (highlighted in white) identified across all environments

toward this. Typically, microbial communities from environments that have a history of NA contamination, such as those found in oil sands and OSPW, can degrade NAs, including recalcitrant NAs, more quickly. Thus, prior exposure to NAs may select for microorganisms capable of more effective NA degradation such as members of the *Proteobacteria*.

In addition to the structure and composition of NAs selecting for various microbial groups, environmental factors such as nutrient limitations, co-occurrence of trace metals, and competition for substrates from other non-NA-degrading microbes are also important drivers in shaping the NA-degrading microbial community. Due to the persistence and toxicity of NAs, there is an urgency to better understand the NA biodegradation pathways, the factors which affect NA degradation and the genes involved. Identification of factors that affect the growth of NA-degrading microorganisms (either enhancing or inhibiting growth), such as the addition of nutrients, aeration, and ozonation, has important implications for the development of more effective NA bioremediation strategies. In conclusion, for future remediation strategies, it may be possible to manipulate environmental conditions to select for only those microbial communities that show higher rates of NA degradation as well as degrade the more persistent NAs.

Acknowledgments We thank Dr. Lisa Gieg, University of Calgary, for her useful comments on the manuscript.

References

Abu Laban N, Dao A, Semple K, Foght JM (2014) Biodegradation of C_7 and C_8 *iso*-alkanes under methanogenic conditions. Environ Microbiol 12:4898–4815

Abu Laban N, Dao A, Foght J (2015) DNA stable-isotope probing of oil sands tailings pond enrichment cultures reveals different key players for toluene degradation under methanogenic and sulfidogenic conditions. FEMS Microbiol Ecol 91:1–12

Aktas Ö, Çeçan F (2007) Bioregeneration of activated carbon: a review. Int Biodeterior Biodegrad 59:257–272

Allard A-S, Neilson AH (1997) Bioremediation of organic waste sites: a critical review of microbiological aspects. Int Biodeterior Biodegrad 39:253–285

An D, Brown D, Chatterjee I, Dong X, Ramos-Padron E, Wilson S et al (2013) Microbial community and potential functional gene diversity involved in anaerobic hydrocarbon degradation and methanogenesis in an oil sands tailings pond 1. Genome 56:612–618

Barrow MP, Headley JV, Peru KM, Derrick PJ (2009) Data visualization for the characterization of naphthenic acids within petroleum samples. Energy Fuel 23:2592–2599

Bataineh M, Scott AC, Fedorak PM, Martin JW (2006) Capillary HPLC/QTOFMS for characterizing complex naphthenic acid mixtures and their microbial transformation. Anal Chem 78:8354–8361

Bauer AE (2015) Enhanced characterization of oil sands acid-extractable organics fractions using electrospray ionization-high-resolution mass spectrometry and synchronous fluorescence spectroscopy. Environ Toxicol Chem 34:1001–1008

Baugh TD, Wolf NO, Mediaas H, Vindstad JE, Grande K (2004) Characterization of a calcium naphthenate deposit- the ARN acid discovery. Prepr Am Chem Soc Div Petrol Chem 49:274

Beddow J, Johnson RJ, Lawson T, Breckels MN, Webster RJ, Smith BE, Rowland SJ, Whitby C (2016) The effect of oil sands process-affected water and model naphthenic acids on photosynthesis and growth in *Emiliania huxleyi* and *Chlorella vulgaris*. Chemosphere 145:416–423

Bordenave S, Kostenko M, Dutkoski M, Grigoryan A, Martinuzzi RJ, Voordouw G (2010) Relation between the activity of anaerobic microbial populations in oil sands tailings ponds and sedimentation of tailings. Chemosphere 81:663–668

Brient JA, Wessner PJ, Doly MN (1995) Naphthenic acids. In: Kroschwitz JI (ed) Encyclopedia of chemical technology, vol 16, 4th edn. Wiley, New York, pp 1017–1029

Canadian Oil Sands Network for Research and Development (CONRAD) (1998) Naphthenic acids background information: discussion report. Canadian Oil Sands Network for Research and Development (CONRAD) Environmental Aquatics Technical Advisory Group (CEATAG). Alta, Edmonton

Chakraborty R, Wu CH, Hazen TC (2012) Systems biology approach to bioremediation. Curr Opin Biotechnol 23:483–490

Chalaturnyk RJ, Scott JD, Ozum B (2002) Management of oil sands tailings. Pet Sci Technol 20:1025–1046

Chambers PA, Culp JM, Glozier NE, Cash KJ, Wrona FJ, Noton L (2006) Northern rivers ecosystem initiative: nutrients and dissolved oxygen-issues and impacts. Environ Monit Assess 113:117–141

Choi J, Hwang G, El-Din MG, Liu Y (2014) Effect of reactor configuration and microbial characteristics on biofilm reactors for oil sands process-affected water treatment. Int J Biodeterior Biodegrad 89:74–81

Clemente JS, Fedorak PM (2005) A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere 60:585–600

Clemente JS, MacKinnon MD, Fedorak PM (2004) Aerobic biodegradation of two commercial naphthenic acid preparations. Environ Sci Technol 38:1009–1016

Clothier LN, Gieg LM (2016) Anaerobic biodegradation of surrogate naphthenic acids. Water Res 90:156–166

Combarros RG, Rosas I, Lavin AG, Rendueles M, Diaz M (2014) Influence of biofilm on activated carbon on the adsorption and biodegradation of salicylic acid in wastewater. Water Air Soil Pollut 225:1858

Del Rio LF, Hadwin AKM, Pinto LJ, MacKinnon MD, Moore MM (2006) Degradation of naphthenic acids by sediment microorganisms. J Appl Microbiol 101:1049–1061

Demeter MA, Lemire J, Yue G, Ceri H, Turner RJ (2015a) Culturing oil sands microbes as mixed species communities enhances *ex situ* model naphthenic acid degradation. Front Microbiol 6:936

Demeter MA, Lemire J, Golby S, Schwering M, Ceri H, Turner RJ (2015b) Cultivation of environmental bacterial communities as multispecies biofilms. In: McGenity TJ, Timmis KN, Nogales B (eds) Hydrocarbon and lipid microbiology protocols. Springer protocols handbooks. Springer, Berlin. https://doi.org/10.1007/8623_2015_82

Dutta TK, Harayama S (2001) Biodegradation of *n*-alkylcycloalkanes and *n*-alkylbenzenes via new pathways in *Alcanivorax* sp. strain MBIC 4326. Appl Environ Microbiol 67:1970–1974

Fall RR, Brown JL, Schaeffer TL (1979) Enzyme recruitment allows the biodegradation of recalcitrant branched hydrocarbons by *Pseudomonas citronellolis*. Appl Environ Microbiol 38:715–722

Fedorak PM, Coy DL, Dudas MJ, Simpson MJ, Renneberg AJ (2003) Microbially-mediated fugitive gas production from oil sands tailings and increased tailings densification rates. J Environ Eng Sci 2:199–211

Foght JM (2015) Microbial metagenomics of oil sands tailings ponds: small bugs, big data. Genome 58:507–510

Foght JM, Fedorak PM, Westlake DWS (1985) Microbial content and metabolic activities in Syncrude tailings pond. AOSTRA J Res 1:139–146

Foght JM, Gieg LM, Siddique T (2017) The microbiology of oil sands tailings: past, present, future. FEMS Microbiol Ecol 93(5):fix034

Folwell BD, McGenity TJ, Price A, Johnson RJ, Whitby C (2015) Exploring the capacity for anaerobic biodegradation of polycyclic aromatic hydrocarbons and naphthenic acids by microbes from oil sands process affected waters. Int J Biodeterior Biodegrad 108:214–221

Folwell BD, McGenity TJ, Whitby C (2016) Characterisation of biofilm and planktonic bacterial and fungal communities transforming high molecular weight polyaromatic hydrocarbons. Appl Environ Microbiol 82:2288–2299

Frank RA, Kavanagh R, Burnison BK, Arsenault G, Headley JV, Peru KM, Solomon KR (2008) Toxicity assessment of generated fractions from an extracted naphthenic acid mixture. Chemosphere 72:1309–1314

Frank RA, Fischer K, Kavanagh R, Burnison BK, Arsenault G, Headley JV, Peru KM, Van Der Kraak G, Solomon KR (2009) Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. Environ Sci Technol 43:266–271

Frankel ML, Bhuiyan TI, Veksha A, Demeter MA, Layzell DB, Helleur RJ, Hill JM, Turner RJ (2016) Removal and biodegradation of naphthenic acids by biochar and attached environmental biofilms in the presence of co-contaminating metals. Bioresour Technol 216:352–361

Frascari D, Bucchi G, Doria F, Rosato A, Tavanaie N, Salviulo R, Ciavarelli R, Pinelli D, Fraraccio S, Zanaroli G, Fava F (2014) Development of an attached-growth process for the on-site bioremediation of an aquifer polluted by chlorinated solvents. Biodegradation 25:337–350

Golby S, Ceri H, Gieg L, Chatterjee I, Marques LLR, Turner RJ (2012) Evaluation of microbial biofilm communities from an Alberta oil sands tailings pond. FEMS Microbiol Ecol 79:240–250

Grewer DM, Young RF, Whittal RM, Fedorak PM (2010) Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? Sci Total Environ 408:5997–6010

Gunawan Y, Nemati M, Dalai A (2014) Biodegradation of a surrogate naphthenic acid under denitrifying conditions. Water Res 51:11–24

Hadwin AKM, Del Rio LF, Pinto LJ, Painter M, Routledge R, Moore M (2006) Microbial communities in wetlands of the Athabasca oil sands: genetic and metabolic characterization. FEMS Microbiol Ecol 55:68–78

Han X, Scott AC, Fedorak PM, Batianeh M, Martin JW (2008) Influence of molecular structure on the biodegradability of naphthenic acids. Environ Sci Technol 42:1290–1295

Headley JV, McMartin DW (2004) A review of the occurrence and fate of naphthenic acids in aquatic environments. J Environ Sci Health A 39:1989–2010

Headley JV, Conly M, Dickson LC, Akve C, Peru KM (2000) Characterization of natural hydrocarbon release from oil sands deposits in tributaries of the Athabasca River Basin, Canada. Can Tech Rep Fish Aquat Sci 2331:89–90

Headley JV, Peru KM, McMartin DW, Winkler M (2002a) Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. J AOAC Int 85:182–187

Headley JV, Tanapat S, Putz G, Peru KM (2002b) Biodegradation kinetics of geometric isomers of model naphthenic acids in Athabasca River water. Can Water Res J 27:25–42

Headley J, Peru K, Adenugba A, Du JL, McMartin DW (2010) Dissipation of naphthenic acids mixtures by lake biofilms. J Environ Sci Health Part A 45:1027–1036

Headley JV, Peru KM, Barrow MP (2016) Advances in mass spectrometric characterization of naphthenic acids fraction compounds in oil sands environmental samples and crude oil - a review. Mass Spectrom Rev 35:311–328

Herman DC, Fedorak PM, Costerton JW (1993) Biodegradation of cycloalkane carboxylic acids in oil sand tailings. Can J Microbiol 39:576–580

Herman DC, Fedorak PM, MacKinnon MD, Costerton JW (1994) Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can J Microbiol 40:467–477

Holowenko FM, MacKinnon MD, Fedorak PM (2000) Methanogens and sulfate-reducing bacteria in oil sands fine tailings wastes. Can J Microbiol 46:927–937

Holowenko FM, MacKinnon MD, Fedorak PM (2002) Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Res 36:2843–2855

Huang J, Nemati M, Hill G, Headley J (2012) Batch and continuous biodegradation of three model naphthenic acids in a circulating packed-bed bioreactor. J Hazard Mater 201–202:132–140

Islam MS, Dong T, Sheng Z, Zhang Y, Liu Y, Gamal El-Din M (2014a) Microbial community structure and operational performance of a fluidized bed biofilm reactor treating oil sands process-affected water. Int J Biodeterior Biodegrad 91:111–118

Islam MS, Dong T, McPhedran KN, Sheng Z, Zhang Y, Liu Y, Gamal El-Din M (2014b) Impact of ozonation pre-treatment of oil sands process affected water on the operational performance of a GAC-fluidized bed biofilm reactor. Biodegradation 25:811–823

Islam MS, Dong T, Zhang Y, McPhedran KN, Liu Y, Gamal El-Din M (2015) Next generation pyrosequencing of microbial biofilm communities on granular activated carbon in treatment of oil sands process affected water. Appl Environ Microbiol 81:4037–4048

Johnson R, Smith BE, Sutton P, McGenity TJ, Rowland SJ, Whitby C (2011) Microbial biodegradation of aromatic alkanoic naphthenic acids is affected by the degree of alkyl side chain branching. ISME J 5:486–496

Johnson RJ, West C, Swaih AM, Folwell BD, Smith BE, Rowland SJ, Whitby C (2012) Aerobic biotransformation of alkyl branched aromatic alkanoic naphthenic acids via two different pathways by a new isolate of *Mycobacterium*. Environ Microbiol 14:872–882

Johnson RJ, Smith BE, Rowland SJ, Whitby C (2013) Biodegradation of alkyl branched aromatic alkanoic naphthenic acids by *Pseudomonas putida* KT2440. Int J Biodeterior Biodegrad 81:3–8

Jones D, West CE, Scarlett AG, Frank R, Rowland SJ (2012) Isolation and estimation of the 'aromatic' naphthenic acid content of an oil sands process-affected water extract. J Chromatogr A 1247:171–175

Kelly EN, Short JW, Schindler DW, Hodson PV, Ma M, Kwan AK, Fortin BL (2009) Oil sands development contributes polycyclic aromatic compounds to the Athabasca River and its tributaries. Proc Natl Acad Sci U S A 106:22346–22351

Kelly EN, Schindler DW, Hodson PV, Short JW, Radmanovich R, Nielsen CC (2010) Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. Proc Natl Acad Sci U S A 107:16178–16183

Kurek J, Kirk JL, Muir DCG, Wang X, Evans MS, Smol JP (2013) Legacy of half a century of Athabasca oil sands development recorded by lake ecosystems. Proc Natl Acad Sci U S A 110:1761–1766

Lai JL, Pinto WS, Kiehlmann E, Bendell-Young LI, Moore MM (1996) Factors that affect the degradation of naphthenic acids in oil sands wastewater by indigenous microbial communities. Environ Toxicol Chem 15:1482–1491

Lemire JA, Demeter MA, George I, Ceri H, Turner RJ (2015) A novel approach for harnessing biofilm communities in moving bed biofilm reactors for industrial wastewater treatment. AIMS Bioeng 2:387–403

Lengger S, Scarlett AG, West CE, Frank RA, Hewitt LM, Milestone CB, Rowland SJ (2015) Use of the distributions of adamantane acids to profile short-term temporal and pond-scale spatial variations in the composition of oil sands process-affected waters. Environ Sci: Processes Impacts 17(8):1415

Lutnaes BF, Krane J, Smith BE, Rowland SJ (2007) Structure elucidation of C_{80}, C_{81} and C_{82} isoprenoid tetracids responsible for naphthenate deposition in crude oil production. Org Biomol Chem 5:1873–1877

Magnusson H, Hanneseth AMD, Sjöblom J (2008) Characterization of C_{80} naphthenic acid and its calcium naphthenate. J Dispers Sci Technol 29:464–473

Marchal R, Penet S, Solano-Serena F, Vandecasteele JP (2003) Gasoline and diesel oil biodegradation. Oil Gas Sci Technol 58:441–448

McKenzie N, Yue S, Liu X, Ramsay BA, Ramsay JA (2014) Biodegradation of naphthenic acids in oil sands process waters in an immobilized soil/sediment bioreactor. Chemosphere 109:164–172

Misiti TM, Tezel U, Tandukar M (2013) Aerobic biotransformation potential of a commercial mixture of naphthenic acids. Water Res 47:5520–5534

Misiti TM, Tezel U, Pavlostathis SG (2014) Effect of alkyl side chain location and cyclicity on the aerobic biotransformation of naphthenic acids. Environ Sci Technol 48:7909–7917

Mollard FPO, Roy MC, Foote AL (2015) Performance of wetland forbs transplanted into marshes amended with oil sands processed water. Environ Monit Assess 187:125

Morii H, Eguchi T, Nishihara M, Kakinuma K, Konig H, Koga Y (1998) A novel ether core lipid with H-shaped C_{80} isoprenoid hydrocarbon chain from the hyperthermophilic methanogen *Methanothermus fervidus*. Biochim Biophys Acta 1390:339–345

Nicolella C, Van Loosdrecht MCM, Heijnen JJ (2000) Wastewater treatment with particulate biofilm reactors. J Biotechnol 80:1–33

Paslawski J, Nemati M, Hill G, Headley JV (2009) Biodegradation kinetics of trans-4-methyl-1-cyclohexane carboxylic acid in continuously stirred tank and immobilized cell bioreactors. J Chem Technol Biotechnol 84:992–1000

Penner TJ, Foght JM (2010) Mature fine tailings from oil sands processing harbour diverse methanogenic communities. Can J Microbiol 56:459–470

Ramos-Padron E, Bordenave S, Lin S, Bhaskar IM, Dong X, Sensen CW, Fournier J, Voordouw G, Gieg L (2011) Carbon and sulfur cycling by microbial communities in a gypsum treated oil sands tailings pond. Environ Sci Technol 45:439–446

Rho EM, Evans WC (1975) The aerobic metabolism of cyclohexanecarboxylic acid by *Acinetobacter anitratum*. Biochem J 148:11–15

Rowland SJ, Scarlett AG, Jones D, West CE, Frank RA (2011) Diamonds in the rough: identification of individual naphthenic acids in oil sands process water. Environ Sci Technol 45:3154–3159

Scarlett AG, Reinhardy HC, Henry TB, West CE, Frank RA, Hewitt LM, Rowland SJ (2013) Acute toxicity of aromatic and non-aromatic fractions of naphthenic acids extracted from oil sands process-affected water to larval zebrafish. Chemosphere 93:415–420

Schramm LL, Stasiuk EN, MacKinnon M (2000) Surfactants in Athabasca oil sands slurry conditioning, flotation recovery, and tailings processes. In: Schramm LL (ed) Surfactants: fundamentals and applications in the petroleum industry. Cambridge University Press, Cambridge, pp 365–430

Scott AC, MacKinnon MD, Fedorak PM (2005) Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. Environ Sci Technol 39:8388–8394

Shuqing Z, Haiping H, Yuming L (2008) Biodegradation and origin of oil sands in the Western Canadian Sedimentary Basin. Pet Sci 5:87–94

Siddique T, Fedorak PM, Foght JM (2006) Biodegradation of short-chain *n*-alkanes in oil sands tailings under methanogenic conditions. Environ Sci Technol 40:5459–5464

Siddique T, Fedorak PM, MacKinnon MD, Foght JM (2007) Metabolism of BTEX and naphtha compounds to methane in oil sands tailings. Environ Sci Technol 41:2350–2356

Siddique T, Penner T, Semple K, Foght JM (2011) Anaerobic biodegradation of longer-chain *n*-alkanes coupled to methane production in oil sands tailings. Environ Sci Technol 45:5892–5899

Siddique T, Kuznetsov P, Kuznetsova A, Li C, Young R, Arocena JM, Foght JM (2014) Microbially-accelerated consolidation of oil sands tailings. Pathway II: solid phase biogeochemistry. Front Microbiol 5:107

Siddique T, Stasik S, Mohamad Shahimin MF, Wendt-Potthoff K (2018) Microbial communities in oil sands tailings: their implications in biogeochemical processes and tailings management. In: McGenity TJ (ed) Microbial communities utilizing hydrocarbons and lipids: handbook of hydrocarbon and lipid microbiology, 2nd edn. Springer, Cham

Singh R, Paul D, Jain RK (2006) Biofilms: implications in bioremediation. Trends Microbiol 14:389–397

Smith BE, Sutton PA, Lewis CA, Dunsmore B, Fowler G, Krane L, Lutnaes BF, Brandal Ø, Sjöblom J, Rowland SJ (2007) Analysis of 'ARN' naphthenic acids by high temperature gas chromatography and high performance liquid chromatography. J Sep Sci 30:275–380

Smith BE, Lewis A, Belt S, Whitby C, Rowland SJ (2008) Effects of alkyl chain branching on the biotransformation of naphthenic acids. Environ Sci Technol 42:9323–9328

Stasik S, Wendt-Pethoff K (2014) Interaction of microbial sulphate reduction and methanogenesis in oil sands tailings ponds. Chemosphere 103:59–66

Stasik S, Loick N, Knöller K, Weisener C, Wendt-Pethoff K (2014) Understanding biogeochemical gradients of sulfur, iron, and carbon in an oil sands tailings pond. Chem Geol 382:44–53

Tan B, Dong X, Sensen CW, Foght JM (2013) Metagenomics analysis of an anaerobic alkane-degrading microbial culture: potential hydrocarbon-activating pathways and inferred roles of community members. Genome 56:599–611

Toor NS, Han XM, Franz E, Mackinnon MD, Martin JW, Liber K (2013) Selective biodegradation of naphthenic acids and probable link between mixture profiles and aquatic toxicity. Environ Toxicol Chem 32:2207–2216

Wang B, Wan Y, Gao Y, Yang M, Hu J (2013) Determination and characterization of oxy-naphthenic acids in oilfield wastewater. Environ Sci Technol 47:9345–9554

West CE, Scarlett AG, Tonkin A, O'Carroll-Fitzpatrick D, Pureveen J, Tegelaar E, Gieleciak R, Hager D, Petersen K, Tollefsen K-E, Rowland SJ (2014) Diaromatic sulphur-containing 'naphthenic' acids in process waters. Water Res 51:206–215

Whitby C (2010) Naphthenic acid biodegradation. Adv Appl Microbiol 70:93–125

Wilde MJ, West CE, Scarlett AG, Jones D, Frank RA, Hewitt LM, Rowland SJ (2015) Bicyclic naphthenic acids in oil sands process water: identification by comprehensive multidimensional gas chromatography-mass spectrometry. J Chromatogr A 1378:74–87

Wilson SL, Li C, Ramos-Padron E, Nesbø C, Soh J, Sensen CW, Voordouw G, Foght J, Gieg LM (2016) Oil sands tailings ponds harbour a small core prokaryotic microbiome and diverse accessory communities. J Biotechnol 235:187–196

Wong ML, An D, Caffrey SM, Soh J, Dong X, Sensen CW, Oldenburg TBP, Larter SR, Voordouw G (2015) Roles of thermophiles and fungi in bitumen degradation in mostly cold oil sands outcrops. Appl Environ Microbiol 81:6825–6838

Wyndham RC, Costerton JW (1981a) Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms within the Athabasca oil sands deposit. Appl Environ Microbiol 41:783–790

Wyndham RC, Costerton JW (1981b) *In vitro* microbial degradation of bituminous hydrocarbons and in situ colonization of bitumen surfaces within Athabasca oil sands deposit. Appl Environ Microbiol 41:791–800

Xue J, Zhang Y, Liu Y (2016) Treatment of oil sands process-affected water (OSPW) using a membrane bioreactor with a submerged flat-sheet ceramic microfiltration membrane. Water Res 88:1–11

Yergeau E, Lawrence JR, Sanschagrin S, Waiser MJ, Korber DR, Greer CW (2012) Next generation sequencing of microbial communities in the Athabasca River and its tributaries in relation to oil sands mining activities. Appl Environ Microbiol 78:7626–7637

Yergeau E, Lawrence JR, Sanschagrin S, Roy JL, Swerhone GDW, Korber DR (2013) Aerobic biofilms grown from Athabasca watershed sediments are inhibited by increasing concentrations of bituminous compounds. Appl Environ Microbiol 79:7398–7412

Yue S, Ramsay BA, Ramsay JA (2015) Biodegradation of naphthenic acid surrogates by axenic cultures. Biodegradation 26:313–325

Fungal Communities in Hydrocarbon Degradation

15

Francesc X. Prenafeta-Boldú, G. Sybren de Hoog, and Richard C. Summerbell

Contents

F. X. Prenafeta-Boldú (✉)
Integral Management of Organic Waste (GIRO), Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Catalonia, Spain
e-mail: francesc.prenafeta@irta.cat

G. S. de Hoog
Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

Center of Expertise in Mycology of RadboudUMC / Canisius Wilhelmina Hospital, Radboud University, Nijmegen, The Netherlands
e-mail: s.hoog@westerdijkinstitute.nl

R. C. Summerbell
Sporometrics Inc, Toronto, ON, Canada

University of Toronto, Dalla Lana School of Public Health, Toronto, ON, Canada
e-mail: rsummerbell@sporometrics.com

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3_8

Abstract

The present chapter reviews and discusses recent advances in the ecophysiology, phylogeny, and biotechnological applications of fungi with respect to their ability to degrade hydrocarbons. There is a very wide fungal biodiversity with diverse enzymatic mechanisms that transform different hydrocarbon chemical structures, from short chain aliphatics to heavy weight polycyclic aromatics. Alkanes and alkylbenzenes are generally metabolized as the sole source of carbon and energy via specialized metabolic pathways that start with the substrate oxidation through cytochrome P450 monoxygenases. Unsaturated alkenes and alkynes, as well as alicyclics, are more recalcitrant to fungal degradation and are often converted to partly oxidized metabolites. Aromatic hydrocarbons ranging from the single benzene ring to the high-molecular-weight polycyclics are generally degraded via one or more of three independent enzymatic systems. The intracellular P450 monooxygenases that detoxify harmful chemicals are universally present in the microsomes of eukaryotic cells, while lignin-degrading fungi specifically produce extracellular peroxidases and laccases that biodegrade aromatic hydrocarbons. Laccases are not exclusively active in lignin biodegradation: other functions have been reported for these enzymes in nonligninolytic fungi. The low functional specificity and high redox potential of peroxidases and laccases enables the oxidation of a broad range of aromatic hydrocarbons and other recalcitrant contaminants. Such co-incidental biodegradation processes often result in partially degraded compounds that do not support fungal growth and that might be more toxic than the parent substrates.

Relevant hydrocarbonoclastic fungal strains deposited in culture collections have been identified and their phylogenies revised and reassessed when necessary. The capacity to assimilate hydrocarbons in fungi may have evolved in the context of biotrophic interactions in environments that are rich in naturally biosynthesized alkanes and volatile alkylbenzenes. The ability to utilize hydrocarbons seems to correlate with virulence toward humans, as seen in phylogenetically unrelated genera of hydrocarbonoclastic fungi, e.g., *Scedosporium* (*Microascales*) and *Exophiala*-*Cladophialophora* (*Chaetothyriales*). Applied research on hydrocarbonoclastic fungi includes studies dedicated to preventing biodeterioration as well as on potential use of the same enzymatic capabilities for bioremediation purposes. Fungal contamination of fuels is a long-standing problem that has acquired new dimensions as new biofuel blends have emerged. Recent improvements in phylogenetic understanding of fungal biodeteriogens may provide enhanced biocontrol opportunities. In work related to restoration of ecosystems, the ability of hydrocarbonoclastic fungi to form extended mycelial networks, in combination with the broad capabilities of their catabolic enzymes, makes these fungi well suited for the bioremediation of hydrocarbon-polluted soils. However, some cases of unsatisfactory biodegradation efficiency in operations conducted at field scale and cases in which toxic intermediates were generated have turned research efforts towards synergistic biodegradation processes mediated by complex microbial populations (i.e., fungal-bacterial mixtures). The assimilatory biodegradation of volatile alkanes and alkylbenzenes by

certain fungal species makes them ideal candidates for the biofiltration of air polluted with these compounds. However, the potential correlation between hydrocarbon utilization and capacity for human infection must be taken into account in the design of biofiltration systems in order to prevent unintended production of biohazardous conditions. Ongoing research is focusing on the precise delimitation of genetic mechanisms that underlie these two apparently converging ecological traits.

1 Introduction

Considering the long history of interrelations between hydrocarbons and living organisms, it is not surprising that biological evolution has resulted in a wide range of metabolic and physiologic adaptations, primarily seen in microorganisms, which serve to cope with and benefit from hydrocarbons. The hydrocarbonoclastic microorganisms include a wide variety of bacteria, archaea, and fungi. The study of their importance in the genesis and biotransformation of petroleum and its derivatives was pioneered in the early 1940s by ZoBell and co-workers, at a time when the biodegradation of hydrocarbons was still contemplated as a "biological curiosity." In his comprehensive 1946 review (ZoBell 1946), he compiled works carried out during the preceding 50 years on the biodegradation, by both bacteria and fungi, of a variety of aliphatic and aromatic hydrocarbons, thus creating the basis of the new discipline of petroleum microbiology (often encompassed within the more general term hydrocarbon microbiology). Ever since, a significant body of literature has been generated on the ecophysiology and metabolism of hydrocarbonoclastic microorganisms, as well as on the ensuing biotechnological implications.

Prokaryotes, especially within the domain *Bacteria*, are able to metabolize a large variety of hydrocarbons using diverse metabolic pathways. They can do so in the presence of a wide range of electron acceptors, environmental conditions, and adaptation strategies to cope with the toxic effect of hydrocarbons (Heider and Schühle 2013; Rosenberg 2013; Sikkema et al. 1995). Bacterial metabolic pathways have often been evolved towards the assimilation of hydrocarbons as the sole source of carbon and energy, usually yielding complete biodegradation into water and carbon dioxide (Fig. 1). This fact and the relatively high turnover rate of bacterial growth makes such microbes ideal biocatalysts for bioremediation purposes. Bacteria primarily display a characteristic form of nutrition, often termed osmotrophy or absorptive heterotrophy, which consists of absorbing soluble substrates from the external environment. Consequently, biodegradation can be hampered when substrates are not bioavailable, i.e., when they cannot cross the cell wall, perhaps because of poor water solubility, or because they are strongly adsorbed onto surfaces within the soil matrix. Metabolic rates of most hydrocarbonoclastic bacteria may also be affected negatively when growth-limiting conditions occur.

Fungi show specific physiological and metabolic advantages over bacteria, since they do not rely solely on soluble, readily absorbed organic compounds for nutrition. They secrete a wide variety of enzymes into their environments that cleave polymeric

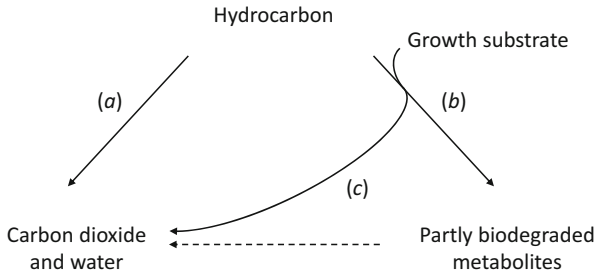

Fig. 1 Schematic representation of the different general metabolic pathways for the biodegradation of hydrocarbons by a specific microorganism (solid rows): assimilatory metabolism (*a*), co-metabolic transformation (*b*), and complete co-metabolic biodegradation (*c*). The complete biodegradation of hydrocarbons by a microbial consortium with at least a second microbial species (dashed row) has also been depicted

substances (Harms et al. 2011). The soluble breakdown products are then absorbed and further catabolized by internal enzymes. Besides the primary fungal oxidizers, many other microorganisms benefit from the released substrates. Complex interactions occur that induce fungi to co-evolve complex strategies, ranging from production of antibiotics on the negative side, to the specific stimulation of indigenous bacteria on the positive side (de Boer et al. 2005; Kohlmeier et al. 2005). Thus, in nature, fungi play a vital role in the recycling of a variety of "recalcitrant" polymeric organic compounds, such as lignin, cellulose, chitin, melanin, and keratin. As a result of this biochemical diversity, the biodegradation of several poorly biodegradable/bioavailable xenobiotics can coincidentally be accomplished with fungi by co-metabolism (Raj et al. 1992). Under such conditions, the parent hydrocarbons can be degraded in varying degrees, from partial transformations to complete biodegradation, but a second organic substrate is often needed to support fungal growth (Fig. 1).

In addition, fungi display a distinctive physiological adaptability during growth by their filamentous thallus (hyphae), a vegetative mode that permits internal translocation of nutrients over solid materials such as soil particles and wood without the need of a liquid phase. Consequently, many fungi are tolerant towards limited water and nutrient availability. They also frequently do well in acidic conditions (Wainwright 1993) that inhibit many bacteria. Yet, despite these advantages and the fact that fungi dominate the living biomass in soil and are also abundant in aqueous systems, they have been less studied and exploited than bacteria in research on the biodegradation of hydrocarbons (Harms et al. 2011).

2 Metabolism of Hydrocarbons by Fungi

Several reviews on the fungal biodegradation of hydrocarbons are available in the scientific literature (Cerniglia and Sutherland 2010; Cerniglia et al. 1992; Haritash and Kaushik 2009; Lindley 1992; Muncnerova and Augustin 1994; Prince 2010;

Sutherland 2003). However, most of these synopses have been focused on certain groups of fungi or specific hydrocarbon breakdown processes, such as the enzymes involved in lignin decay and in the detoxification of xenobiotics, which have primarily been investigated in relation to the biodegradation of PAHs. The recent years have witnessed the emergence of new fungal biosystematic concepts and newly understood metabolic functions that have yielded information challenging previous assumptions. An example is seen in new data on the assimilatory metabolism of certain aromatics and alkylbenzenes. Molecular phylogeny has improved our understanding of the identities of various hydrocarbonoclastic fungal species. Meaningful species groupings in turn increase our knowledge of the ecophysiology and evolution of hydrocarbon metabolism in fungi, as well as the viability and biosafety of the associated biotechnological applications.

2.1 Aliphatics and Alicyclics

The growth of certain fungi on paraffins, i.e., mixtures of alkanes containing between twenty (eicosane) and forty (tetracontane) carbon atoms (C20-C40), was already recognized at the beginning of the twentieth century (ZoBell 1946). Ascomycetes within the genera *Penicillium* and *Aspergillus* were the most commonly reported species at that time. Interest in the fungal biodegradation of aliphatics increased markedly in the 1960s with the advent of jet aircraft, which resulted in a shift from gasoline to kerosene-based fuels. Some aircraft accidents were attributed to fungal mats that clogged the fuel supply to engines (Parbery 1971). These fungi grew in the oil/water interface that accumulated inside fuel storage tanks as a result of water condensation. They utilized kerosene aliphatics in the C10-C20 range as sources of carbon and energy. The fungus most frequently found in kerosene tanks was *Amorphotheca resinae*, long known in pre-2013 dual fungal nomenclature by the asexual state name *Hormoconis resinae* and, in earlier decades, as *Cladosporium resinae*. Because of its recurrent reporting in aviation fuels, but also from creosoted wood, this fungus earned the informal naming "kerosene fungus" or "creosote fungus." Cultivation under laboratory conditions demonstrated that *A. resinae* generally grows on intermediate chain length *n*-alkanes (C9-C14), but the substrate specificity among isolates was variable (Cofone et al. 1973). To this date, a very wide diversity of fungal genera reported from fuel tanks has been compiled in subsequent literature reviews: *Acremonium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botrytis*, *Candida*, *Cephalosporium* (=*Acremonium*), *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Curvularia*, *Drechslera*, *Epicoccum*, *Geomyces*, *Geotrichum*, *Gliomastix*, *Fusarium*, *Hansenula*, *Helminthosporium* (most probably = *Drechslera*), *Humicola*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Phialophora*, *Phoma*, *Phomopsis*, *Pseudallescheria* (=*Scedosporium*), *Rhinocladiella*, *Rhizopus*, *Rhodotorula*, *Saccharomyces*, *Sordaria*, *Stemphylium*, *Thielavia*, *Trichoderma*, *Trichosporon*, *Trichothecium*, *Tritirachium*, and *Ulocladium* (Gaylarde et al. 1999; Yemashova et al. 2007). However, presence does not necessarily imply a relevant metabolic capacity and fungi able to grow on

hydrocarbons are restricted to specific phylogenetic groups that may be adapted to break down distinctive arrays of hydrocarbon structures.

Pioneering works on the assimilation of gaseous *n*-alkanes, from methane to butane (C1-C4), suggested that this feature could be relatively common in fungi (Davies et al. 1973). Utilization of methane as the sole carbon and energy source was demonstrated in five yeasts identified as *Sporobolomyces* and *Rhodotorula* spp. (*Basidiomycota*, *Sporidiobolales*) on physiological grounds, but their growth on methane was very slow, with generation times that lasted a week or longer (Wolf and Hanson 1980). Unfortunately, these strains appear not to have been preserved, and subsequent reports on methane assimilation by fungi remain dubious. Representatives of the genera *Scedosporium* and *Graphium*, the latter probably a synanamorph of the former, have also been described to grow on gaseous alkanes, with the exception of methane (Davies et al. 1973; Onodera et al. 1990). These fungi were therefore proposed as bioindicators for the prospecting of natural gas and as catalysts in single-cell protein projects (Lindley 1992). The preservation of the thoroughly studied *Graphium* strain CBS 116421 (ATCC 58400) and phylogenetic updates through molecular methods demonstrated that it actually belongs to the species *Scedosporium boydii* (*Ascomycota*, *Microascales*). This fungus has been studied concerning its capacity to grow on several *n*-alkanes in the size range of gasoline (C5-C9) and diesel (C10-C20) (April et al. 1998; Janda-Ulfig et al. 2008).

Assimilation of higher *n*-alkanes (>C9) is widespread among ascomycetous and basidiomycetous yeasts in the orders *Saccharomycetales* and *Trichosporonales*, respectively (Table 2). Reports on alkane utilization are recurrent for *Candida*, *Cryptococcus*, *Trichosporon*, and *Yarrowia* species, particularly on *n*-hexadecane (C16), one of the most abundant hydrocarbons in diesel and kerosene fuels and often used as model substrate for microbial growth. The biochemistry, genetics, and biotechnology of alkane assimilation have long been studied and reviewed for *Yarrowia lipolytica* (*Candida oleophila*), a species with great interest in industrial microbiology (Barth and Gaillardin 1996; Beopoulos et al. 2010; Fickers et al. 2005). Assimilation of phenolic compounds, but not aromatic hydrocarbons, was also verified in certain cases (Chrzanowski et al. 2008; Sorkhoh et al. 1990). Some of the latter yeasts occurred in relation to anthropogenic hydrocarbon pollution, but, interestingly, others were isolated from natural environments like rotten wood and mushrooms, as well in association with insects and nematodes (Table 2). A specific mechanism for cell adhesion to chitin has recently been described for *Candida albicans* (Ishijima et al. 2017). Assimilation of higher alkanes appears to be a common feature in entomopathogenic fungi within the *Clavicipitaceae* (*Hypocreales*), such as *Metarhizium anisopliae* and *Beauveria bassiana*. It is also found in the related nematophagous fungus *Paecilomyces lilacinus* (Table 2), which is now reclassified as *Purpureocillium lilacinum* (*Ophiocordycipitaceae*, *Hypocreales*). Alkane metabolism in these fungi has been shown to be associated with the biosynthesis of certain lipid metabolites (Napolitano and Juárez 1997), as well as to the excretion of hydrophobins enhancing the adhesion of conidia on the cuticles of insect and nematode hosts (Vigueras et al. 2014).

The metabolic pathways proposed for the assimilation of aliphatics by fungi require the occurrence of substrate oxidation in microsomes, a process mediated by cytochrome P450 (*CYP*) monooxygenases (Rehm and Reiff 1981). These enzymes belong to the hemeprotein superfamily and play a central role in the oxidative metabolism of endogenous metabolites in all life domains (Lamb et al. 2009). *CYP* monooxygenases are, in general, the terminal oxidases in electron transfer chains coupled to NADPH reductases, which supply the electrons for the insertion of one atom of oxygen into the aliphatic chain, while the other oxygen atom is reduced to water (Chen et al. 2014). Following alkane oxidation to a primary alcohol, further oxidations produce aldehydes and finally fatty acids. This is the most frequently encountered mechanism, but two variants have been described (Fig. 2): diterminal oxidation to form dicarboxylic acids and subterminal oxidation to secondary alcohols. The most common biodegradation pathway for alkanes reported in fungi starts with the hydroxylation of the terminal methyl group. The resulting fatty acids are then incorporated to the central catabolic pathways via *p*-oxidation, involving the initial activation of the fatty acid to form an acyl-CoA ester. Gaseous

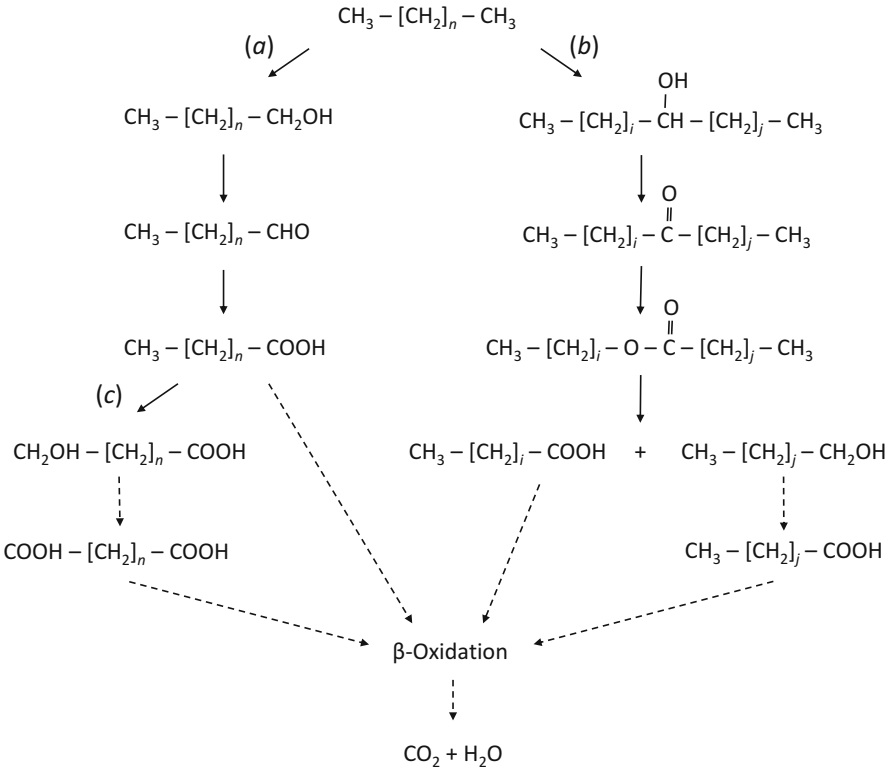

Fig. 2 Metabolic pathways for the assimilation of *n*-alkanes by fungi showing the terminal (*a*), subterminal (*b*), and diterminal (*c*) oxidation variants (Boulton and Ratledge 1984)

n-alkenes and *n*-alkynes were reported as selective inhibitors of the *CYP* monooxygenase responsible for the initial *n*-alkane oxidation: growth of *Scedosporium boydii* ATCC 58400 on ethane was blocked when the fungus was exposed to low concentrations of propene and propyne (Curry et al. 1996). The activity of an alkane-specific *CYP* monooxygenase in this fungus has recently been demonstrated via RNAi silencing (Trippe et al. 2014).

In addition to the pathways mentioned above, unsaturated and branched-chain aliphatics, as well as alicyclics, can be oxidized by fungi via a number of routes yielding epoxides, alcohols, diols, and carboxylic acid units. These reactions usually do not support fungal growth and alicyclics are generally more recalcitrant to biodegradation than aliphatics (Sutherland 2003). Yet, the literature on the fungal metabolism of these compounds is almost nonexistent. A fungus identified as *Ophiostoma* sp. that was isolated from an air biofilter, through which vapors of the bicyclic monoterpene *a*-pinene were being passed, was able to use this compound as the sole source of carbon and energy (Jin et al. 2006). Anamorphs in *Ophiostoma* have generally been treated under the genus *Sporothrix*, which has also been linked to the assimilation of alkylbenzenes (see Sect. 2.3). A recent report described the partial oxidation by *Candida* and *Trichosporon* yeasts of cyclohexane to cyclohexanone as a terminal product, (Dallinger et al. 2016). Cyclohexanone serves as growth substrate for the fungus *Exophiala jeanselmei* (Hasegawa et al. 1990), which belongs to the group of the black yeasts discussed further below. Poor biodegradation has been reported for the significantly larger alicyclic structures of steranes like cholestane by axenic fungal cultures (Mulheirn and Van Eyk 1981). In conclusion, the complete biodegradation of more complex hydrocarbon structures often requires synergistic interactions among multiple fungi and bacteria.

2.2 Aromatics

The fungal biodegradation of aromatic hydrocarbons, particularly the more recalcitrant PAHs, has been reviewed extensively in recent years by investigators focusing on detoxification and bioremediation (Cerniglia and Sutherland 2010; Ghosal et al. 2016; Harms et al. 2011; Kadri et al. 2017; Kennes and Veiga 2012). Three very different – yet not mutually exclusive – enzymatic mechanisms have generally been proposed for the breakdown of aromatics: oxidation of the aromatic ring by intracellular *CYP* monooxygenases for detoxification, the nonspecific and coincidental oxidative action of extracellular aromatics by excreted lignin-degrading peroxidases, and the same kind of coincidental action involving extracellular laccases (Table 1). All these metabolic processes are primarily of a co-metabolic nature, which means that aromatics do not support fungal growth and are usually converted to partly oxidized intermediates (Fig. 1). These metabolites are generally less toxic than is the original substrate but, depending on the biotransformation process that occurs; they can also, by chance, be more toxic than the parent hydrocarbon. This situation is termed “biomagnification.”

Table 1 Fungal enzymes involved in the biodegradation of aromatic hydrocarbons

Enzyme (Enzyme Commission no.)	Reaction mechanisms	Main function (occurrence)	Redox potential (V)	Review references
Cytochrome P450 monooxygenases	O_2-dependent monooxygenation (epoxidation and hydroxylation) of the aromatic ring with release of H_2O	Detoxification of xenobiotics (microsomes)	–	(Chen et al. 2014, van den Brink et al. 1998)
Lignin peroxidases (1.11.1.14)	H_2O_2-dependent one-electron direct oxidation of the aromatic ring	Lignin degradation (extracellular)	1.4–1.5	(Reddy and D'Souza 1994)
Manganese peroxidases (1.11.1.13)	H_2O_2-dependent one-electron oxidation of Mn^{2+} to Mn^{3+}, which then oxidizes the aromatic ring	Lignin degradation (extracellular)	1.0–1.2	(Hofrichter 2002)
Versatile peroxidases (1.11.1.16)	H_2O_2-dependent one-electron oxidation of the both the aromatic ring and Mn^{2+} to Mn^{3+}	Lignin degradation (extracellular)	1.4–1.5	(Ruiz-Dueñas et al. 2009)
Laccases (1.10.3.2)	O_2-dependent one-electron oxidation of the aromatic ring in the presence of redox mediators	Lignin degradation, melanin synthesis (extracellular)	0.4–0.8	(Baldrian 2006)

2.2.1 Cytochrome P450 Monooxygenases

Since the last quarter of the twentieth century, Cerniglia and coworkers have contributed to a significant body of literature on the biotransformation of aromatic hydrocarbons and related xenobiotics into partly oxidized metabolites by the zygomycete *Cunninghamella elegans* (Cerniglia and Sutherland 2010). Species in the genus *Cunninghamella* are able to metabolize a wide variety of xenobiotics using both phase I (oxidative) and phase II (conjugative) biotransformation mechanisms (Fig. 3) (Zhang et al. 1996). The *CYP* system catalyzes the ring-epoxidation of the aromatic structure to form arene oxides, which can either undergo enzymatic hydration by epoxide hydrolase to *trans*-dihydrodiols, or else rearrange nonenzymatically to form phenols (Sariaslani 1991). Arene epoxides that are formed at the "bay-region" – that is, between angled benzene rings – of PAHs like benzo[*a*]pyrene are known to be highly reactive and to function as carcinogens (Juhasz and Naidu 2000). Hydroxylation products can undergo further detoxification by O-conjugation to various intermediate forms, including methyl, glucoside, glucuronide, sulfate, and xyloside forms. These molecules are more soluble and can eventually be excreted. In general, these biotransformations occur in regio- and stereo-selective manners producing outcomes similar to those seen in mammalian enzyme systems. For this reason, *Cunninghamella* species are regarded as model organisms for understanding the detoxification of metabolites derived from a wide diversity of xenobiotics, including drugs (Asha and Vidyavathi 2009).

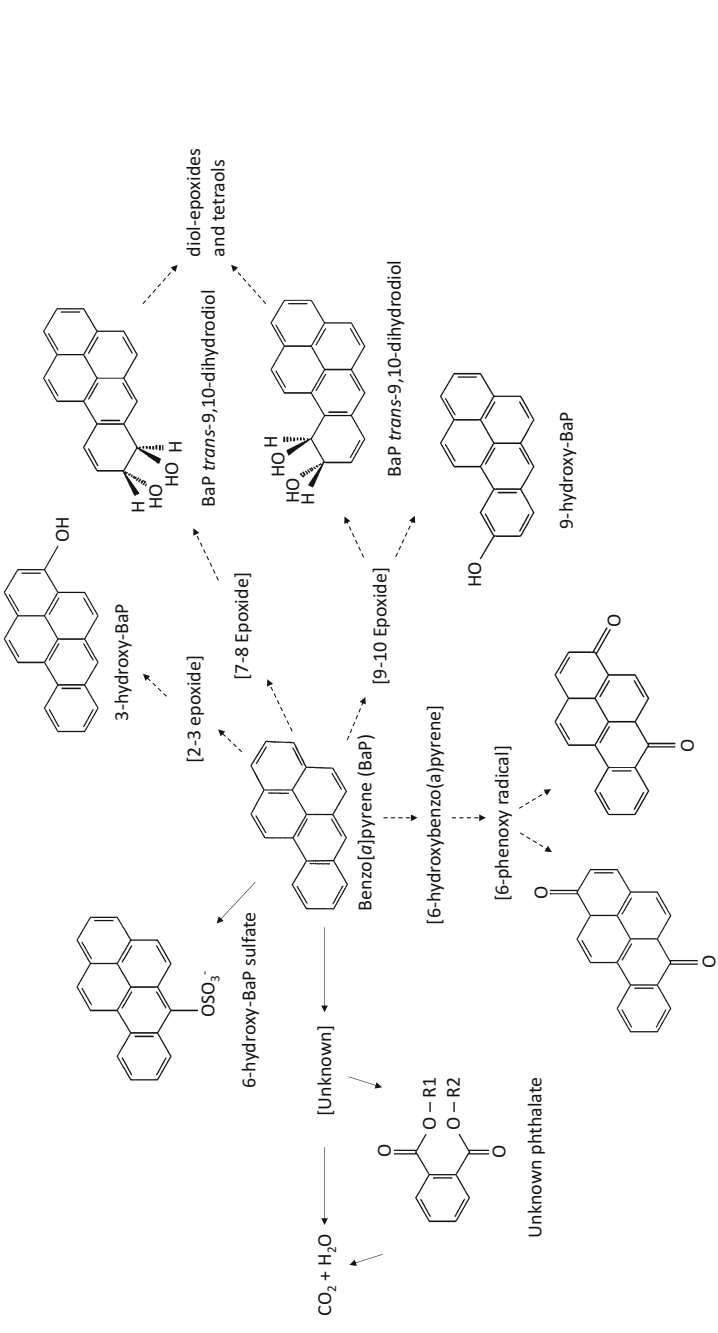

Fig. 3 Co-metabolic and assimilative metabolic pathways used in the biodegradation of benzo[*a*]pyrene by the fungi *Cunninghamella elegans* (dashed arrows) and *Fusarium solani* (solid arrows). As yet undetected, putative metabolites are indicated in brackets. (Adapted from Cerniglia et al. (1992) and Rafin et al. (2008))

Species of *Cunninghamella* have also been investigated in bioremediation efforts, but the data so far are limited (Tomaselli Scotti and Durand 2000). Yet, a renewed interest in the importance of *CYP* monooxygenases in bioremediation has arisen in recent years. A versatile *CYP* monooxygenase able to oxidize PAHs made up of four to six rings, and also able to act on long-chain *n*-alkanes (C9-C12, C15-C19), has been described in the basidiomycete *Phanerochaete chrysosporium* (Syed et al. 2013). This enzyme was characterized by possessing an active-site cavity that was unusually large compared to other hydrocarbon-oxidizing *CYP* analogues from mammals and bacteria. This feature would explain the enzyme's extraordinarily broad substrate specificity. It is important to notice that *P. chrysosporium* is a well-known white-rot fungus that is also capable of oxidizing several PAHs by the action of unrelated lignin peroxidases and laccases. It has been extensively investigated for bioremediation, as discussed in Sect. 2.2.2.

Interestingly, *CYP* monooxygenases appeared to play a fundamental role in the assimilation of benzo[*a*]pyrene as sole carbon and energy source by the ascomycete *Fusarium solani* strain F33 (UCEIV-S19, Table 3). This fungus was isolated from compost after enrichment with domestic fuel oil. Growth on benzo[*a*]pyrene was substantiated in axenic culture using a basal mineral medium plus this compound and was confirmed by showing significant recovery of biomass and substrate ^{14}C-radiolabel as ^{14}C-CO_2 at the end of incubation. This process was hindered by the addition of 1-aminobenzotriazol, a specific *CYP* monooxygenase inhibitor. However, subsequent metabolic profiling suggested that two different biodegradation pathways might be involved (Fig. 3), one being a detoxification process mediated by phase I *CYP* monooxygenases and phase II aryl sulfatases and the other being the fission of the aromatic ring by lignin peroxidases and laccases, followed by free radical attack (Rafin et al. 2008). These processes are discussed in more detail below.

2.2.2 Lignin Peroxidases

Four different types of oxidase enzymes are principally involved in the ligninolysis (Table 1): Two of these are Mn-dependent (*MnP*) and Mn-independent peroxidases; the latter are known as versatile peroxidases (*VP*). Also involved are glycosylated heme-containing lignin peroxidases (*LiP*) and copper-containing phenol-oxidizing laccases (*Lac*). The last of these will be discussed in more detail in the following section. Detailed molecular and enzymatic mechanisms of *MnP* and *LiP* have been reviewed elsewhere (Hofrichter 2002; Reddy and D'Souza 1994). In overview, in the presence of endogenously formed H_2O_2, *LiP* oxidizes veratryl alcohol, an endogenously generated low-molecular mass redox mediator, which in turn carries out one-electron oxidations of nonphenolic aromatics to form aryl cation radicals. These radicals initiate a chain of random oxidative chemical reactions that result in a variety of aliphatic and aromatic oxidized products. *MnP* performs an H_2O_2-dependent oxidation of Mn^{2+} to Mn^{3+}, which is stabilized by fungal chelators such as oxalic acid. Chelated Mn^{3+} then acts as a low-molecular mass redox-mediator that attacks phenolic structures, while other recalcitrant aromatic compounds are oxidized via cation radicals. *VP* enzymes differ from *MnP* by its Mn-independent activity. Several homologous and heterologous genes encoding for *LiP* and *MnP*

have been sequenced, and their expression is regulated at the mRNA level by nitrogen and, in the case of *MnP*, by Mn^{2+} and by heat shock (Gold and Alic 1993).

The biodegradation of lignin has been primarily linked to a specialized group of wood-decaying basidiomycetous fungi and a few ascomycete genera within the *Xylariaceae* family that degrade lignin and give a characteristic bleached appearance to wood. This appearance of the substrate has caused them to be named as "white-rot" fungi. Lignin degradation occurs as part of the process of gaining access to cellulose and hemicellulose, substrates that are used effectively as carbon and energy sources by ligninolytic fungi. White-rot fungi have commonly been selected as model organisms for the degradation of aromatic pollutants. Species tested include members of the genera *Phanerochaete*, *Bjerkandera*, and *Trametes*, in the order *Polyporales* (Muncnerova and Augustin 1994). Lignin degradation has also been reported in non-white-rot fungi that are usually associated to the decomposition leaf litter in soil (Osono 2007; Steffen et al. 2007). The litter-decomposing basidiomycete genera that are most frequently studied for biodegradative capability with aromatic hydrocarbons include *Agrocybe*, *Clitocybe*, *Collybia*, *Marasmius*, *Mycena*, and *Stropharia*. Even though significant biodegradation into carbon dioxide has been reported in certain cases, aromatic hydrocarbons and lignin are only degraded by co-metabolism in litter-decomposing fungi. *MnP* and *Lac*, rather than *LiP*, appeared to be the key enzymes in the biodegradation process, which is enhanced upon Mn^{2+} supplementation.

Though ligninolytic activity is significantly lower in the ascomycetes than in the basidiomycetes, it has also been reported in soil and litter fungi belonging to the cosmopolitan genera *Aspergillus*, *Penicillium*, and *Fusarium* (Rodriguez et al. 1996). Low levels of *MnP* and *VP* activities (but not *LiP*) were detected in certain isolates of *Fusarium solani* during growth on benzo[*a*]pyrene (Saparrat et al. 2000). Along with *CYP*, these enzymes might play a significant role in the assimilation of benzo[*a*]pyrene by this fungus (Rafin et al. 2000). Fungal *CYP* monooxygenases also play a fundamental role in the further oxidation of lignin break-down products into phenols, aromatic acids, and their methoxylated or reduced analogues (Syed et al. 2013). The methoxylated and reduced analogue compounds have significant water solubility and are catabolized intracellularly by a relatively wide diversity of fungi and also bacteria, mainly soil saprobes (de Boer et al. 2005).

2.2.3 Laccases

Laccases are copper-containing oxidase enzymes that act on organic compounds by performing one-electron oxidations in an O_2-dependent reaction, generating free radicals from different low-molecular mass redox mediators (Baldrian 2006). Besides playing a role in lignin degradation, laccases have a vital function in the formation of fungal melanins (Mayer and Staples 2002), where they promote the oxidative coupling of phenolic monomers. As with peroxidases, laccases are able to oxidize a wide range of aromatic compounds, including hydrocarbons. The laccase-mediated biodegradation of mixtures of benzene, toluene, ethylbenzene, and xylene (BTEX) has been described with the ligninolytic fungus *Phanerochaete chrysosporium*. Significant biodegradation to carbon dioxide was reported by

isotopic labeling studies, but interestingly, lignin peroxidases were apparently not involved in that process (Yadav and Reddy 1993). A more recent study on BTEX biodegradation using *Trametes versicolor* demonstrated that these substrates were all oxidized by extracellular hydroxyl radicals induced by incubating the fungus with 2,6-dimethoxy-1,4-benzoquinone (DBQ) and Fe^{3+}–EDTA. Under these conditions, hydroxyl radicals were generated through DBQ redox cycling catalyzed by quinone reductases and laccases (Aranda et al. 2010). In fact, previous studies on *T. versicolor* had already shown that laccases from this fungus oxidize several PAHs (Majcherczyk et al. 1998). Addition of the redox mediators 1-hydroxybenzotriazole (HBT) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) to the reaction mixture increased the oxidation rates of PAHs.

Interestingly, as reviewed recently, it has been demonstrated that fungal laccases from nonligninolytic fungi can oxidize PAHs as well (Marco-Urrea et al. 2015). This finding has expanded the possibility of using various alternative fungal species, some of which might be better adapted to a given set of the bioremediation conditions. Many of these PAH oxidation studies have been performed with representatives of the ascomycetous genus *Fusarium*, in the order *Hypocreales* (Wu et al. 2010). With about 1000 described species, this diverse genus is widely distributed in soil and associated with plants as harmless saprobes but also as pathogens. Several laccase genes have been identified and characterized in the genome of the wilt fungus *Fusarium oxysporum*. Some of them were constitutively expressed in culture, whereas others appeared up-regulated when expressed during the plant infection process (Cañero and Roncero 2008).

The production of extracellular laccases, up to levels that are comparable to those of ligninolytic fungi, has also been observed in the so-called black yeast-like fungi (Fig. 4). This functional group of relatively poorly understood ascomycetes owes its name to the strongly melanized thallus and the ability of some species to produce masses of budding cells, along with filaments and meristematic structures (de Hoog 1999). Such physiological flexibility and strong melanin pigmentation enables these organisms to colonize a wide range of hostile and sometimes, very unusual environments, so that many species are considered to be polyextremophilic. Black yeasts primarily belong to two clearly delimited orders: the *Dothideales* and the *Chaetothyriales*. Despite the similarities in morphology between these two orders, there are some significant differences at physiological and ecological level.

Dothidealean species tend to be isolated from the environment featuring conditions such as extreme pH and temperature, high salinity, radiation, or desiccation (de Hoog 1999). The biodegradation of PAHs by laccases secreted from dothidealean black yeasts has been verified for the ubiquitous species *Aureobasidium pullulans* and has also been found in an equally cosmopolitan, remote relative, the capnodialean *Cladosporium sphaerospermum* (Leelaruji et al. 2014; Potin et al. 2004). The phylogenetically related *Hortaea acidophila* was isolated from an extract of brown coal that, besides holding PAHs, must have been rich in humic and fulvic acids (Hölker et al. 2004). This fungus grew at a pH as low as 0.6 under laboratory conditions. Follow-up genetic and physiological studies demonstrated that it produces two different laccases (Tetsch et al. 2006). The first is intracellular and active

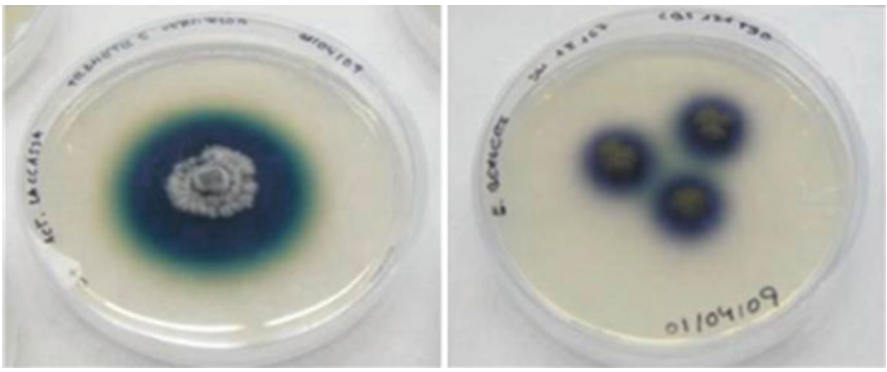

Fig. 4 Growth of the ligninolytic fungus *Trametes versicolor* (left) and the black yeasts *Exophiala bergeri*, order *Chaetothyriales* (right) chromogenic agar plates amended with ABTS. Dark halos are indicative of extracellular laccase activity

at physiological conditions; it is involved in melanin synthesis. Interestingly, the second laccase is extracellular and exhibits high stability under acidic conditions, with an optimum pH for activity of only 1.5. Its function, then, might be related to the hydrolysis of the coal matrix as a step in the assimilation of break-down products. The biotechnological potential of such extremozymes remains to be explored. Chaetothyrialean fungi can also be present in extreme environments, but they are often isolated from sites that are polluted with monoaromatic hydrocarbons. Examples include BTEX-polluted soils in gasoline stations (Prenafeta-Boldú et al. 2001a), gasoline supply appliances (Isola et al. 2013), and creosoted wood (Gümral et al. 2014). Species within this group are also known to produce extracellular laccases (Fig. 4), but these enzymes have yet to be investigated. Recent full genome characterization studies on representatives of this group have revealed the presence of several laccase genes (Moreno et al. 2017).

2.3 Alkylbenzenes

Fungal growth on *n*-alkylbenzenes as the sole carbon and energy source was first described in the 1980s for some ascomycetes (*Beauveria*, *Verticillium*, *Paecilomyces*, and *Penicillium* spp.) isolated from oil-polluted environments (Fedorak and Westlake 1986). Minimum side-chain lengths from C4 to C9, depending upon the strain, were required for growth. Unfortunately, those first alkylbenzene-growing isolates were not preserved in fungal collections and appear to have been lost to further research. A decade later, alkylbenzene assimilation was claimed for a number of fungal isolates from air biofilters treating styrene (Cox et al. 1993a): *Gliocladium roseum* CBS 102.94, *Exophiala jeanselmei* CBS 238.93, and a number of unpreserved *Penicillum* spp. Subsequent detailed studies on the assimilation of toluene and styrene were performed with *Cladosporium sphaerospermum*

CBS 114326 and *Exophiala jeanselmei* CBS 658.76, respectively (Cox et al. 1996; Weber et al. 1995). Updated molecular phylogeny showed that CBS 102.94 and CBS 238.93 actually belong to *Bionectria ochroleuca* and *Cyphellophora sessilis*. The strain CBS 114326 was confounded with a second isolate (now probably lost) that belonged to *Cladophialophora saturnica* (Badali et al. 2009), while the identity of CBS 658.76 corresponded in fact to *Exophiala oligosperma* (de Hoog et al. 2003).

Since those first reports, the number of isolated fungi with a proven capacity to assimilate alkylbenzenes as the sole source of carbon and energy has been increasing (Table 3). The most commonly reported growth substrates are toluene, ethylbenzene, and styrene. Xylene isomers appeared to be partly oxidized in BTEX mixtures while benzene was not biodegraded (Prenafeta-Boldú et al. 2002). It has been claimed that black yeasts and related fungi can also use benzene as growth substrate (Cofone et al. 1973; Qi et al. 2002). Problematically, this statement was based only on the qualitative observation of fungal biomass developing upon exposure of the inoculum to benzene via the gas phase. These experiments were interpreted without the support of a growth curve. Given the oligotrophic nature of the black yeasts, these results must be taken with caution. Many of the isolates with a known capacity to grow on alkylbenzenes were obtained from biofilters treating air streams polluted with toluene and styrene (Table 3). Others were isolated via selective enrichment procedures, such as the technique shown in Fig. 5. Biofilter conditions can be simulated by incubating fungi onto moistened, inert packing material under hydrocarbon-enriched, controlled atmospheres (Prenafeta-Boldú et al. 2001a). An oil flotation isolation technique has also been applied successfully to recover fungi growing on hydrocarbons from a variety of environments (Satow et al. 2008). With this technique, dilute saline solution overlaid by mineral oil is used as to select for the hydrophobicity and the hydrocarbonoclastic capacities of fungi that can grow in the oil. Both enrichment methods tend to select for melanized fungi within the *Chaetothyriales*.

In bacteria, assimilation of alkylbenzenes is carried out by three types of oxidative reactions: (i) the successive mono-oxidation of bonds in the aromatic ring to form first alkyl-phenols and then alkyl-catechols, (ii) the direct di-oxidation of the aromatic ring to form alkyl-catechols, and (iii) the oxidation of the alkyl substituent to form aromatic alcohols that are further oxidized to carboxylic acids, prior to hydroxylation of the ring to form catechols. The aromatic ring is then cleaved either at the ortho or the meta position and the generated muconates are incorporated to the Krebs cycle (Gibson and Subramanian 1984). All available evidence on the fungal assimilation of alkylbenzenes points to use of this last pathway (Fig. 6), where the initial oxidation of the alkyl group leads to the formation of aromatic acids, which are eventually assimilated (Cox et al. 1996; Prenafeta-Boldú et al. 2001b, 2002; Weber et al. 1995). The initial oxidative attack is carried out by specific *CYP* monooxygenases (Cox et al. 1996; Luykx et al. 2003). This metabolic route has recently been validated at the transcriptional level on toluene-growing cells of *Cladophialophora immunda* (Blasi et al. 2017). Such a conserved pathway for the assimilation of alkylbenzenes would explain the absence of known fungal isolates with a proven capacity to grow on benzene, which can only be broken down via

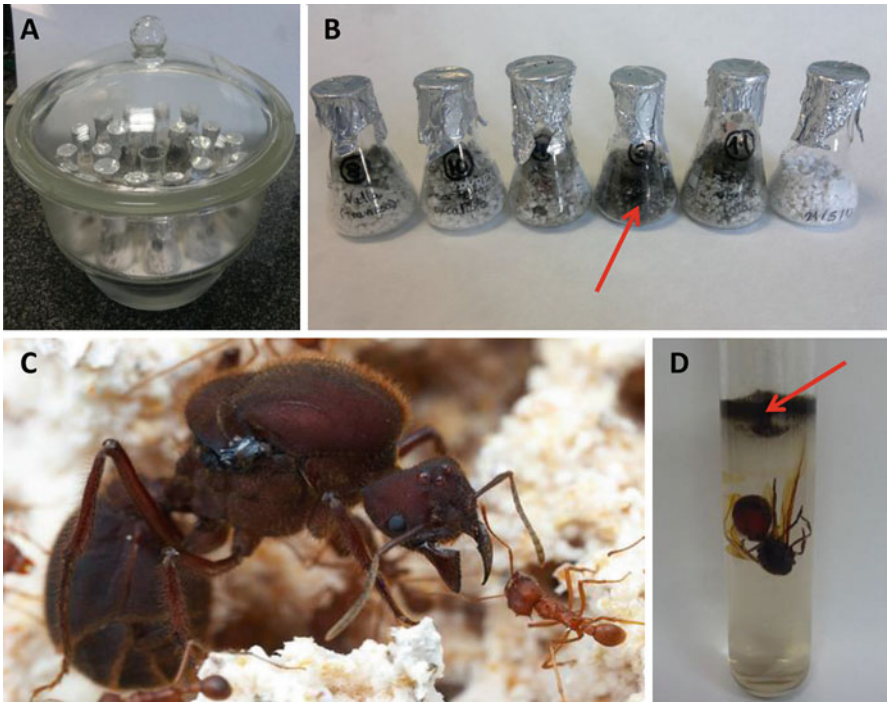

Fig. 5 Selective enrichment of hydrocarbonoclastic fungi from soil samples upon solid state-like incubations under a controlled atmosphere (**a**) using perlite granules as support material (**b**), and from leaf-cutting ants (**c**) by means of the oil flotation technique (**d**). After several days/weeks of incubation, significant growth of melanized fungi (red arrows) is evident on the mineral oil and on the perlite from certain batches. (Images **c** and **d** have kindly been provided by D. Attili-Angelis and F. C. Pagnocca)

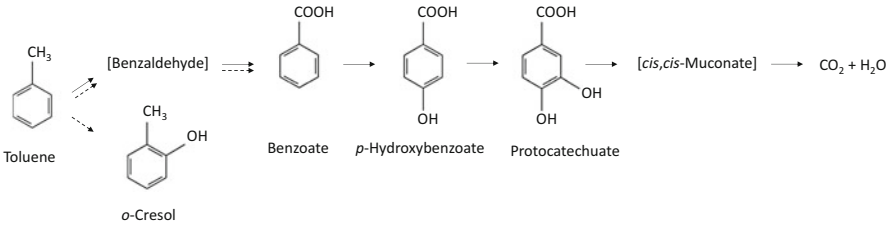

Fig. 6 Assimilatory and co-metabolic pathways for the biodegradation of toluene by fungi using this substrate as the sole source of carbon and energy (solid arrow) and by *Cunninghamella elegans* (dashed arrows). Nondetected putative metabolites have been indicated between brackets. (Adapted from Prenafeta-Boldú et al. 2001b)

direct oxidation of the aromatic ring. Interestingly, Blasi et al. (2017) also claimed that some of the genes involved in the assimilation of toluene by *C. immunda* might have originated from interdomain horizontal gene transfer events with bacteria in the *Pseudomonas* group.

The assimilation of alkylbenzenes differs from the previously described forms of coincidental biodegradation in that it must have evolved from selective pressures derived from contact with those substrates. This type of metabolic capacity appears most commonly in certain lineages from the *Chaetothyriales*, *Hypocreales*, and *Ophiostomales*, as proven alkylbenzene-degrading isolates representing these orders have been isolated independently from multiple sources (Table 3). Reported natural niches for the hydrocarbonoclastic *Chaetothyriales*, particularly in the *Cladophialophora* and *Exophiala* genera, comprise decaying wood and bark (Badali et al. 2008), ripening fruits (Nascimento et al. 2017), and leaf-cutting ants (Duarte et al. 2014; Voglmayr et al. 2011). These environments produce a number of volatile hydrocarbons that may be involved in signaling or may be derived from the decomposition of lignocellulosic organic matter. Concerning the order *Hypocreales*, the *Paecilomyces* hydrocarbonoclastic strains, some biofilter isolates were initially ascribed to *P. variotii* (Estevez et al. 2005; García-Peña et al. 2008) but have subsequently been deposited as *P. sinensis* CBS 113409 and CBS 115145. Based on recent morphological and molecular data, this latter species has been reclassified with the new combination *Polycephalomyces sinensis* in the family *Ophiocordycipitaceae* (Wang et al. 2012). Interestingly, all known species in this newly defined genus are associated with insects or entomogenous fungi. Similarly, *Bionectria ochroleuca* is a well-known mycoparasite of fungi and nematodes and has been reported to be entomopathogenic as well (Toledo et al. 2006). Fungi in the *Ophiostomales*, i.e., *Sporothrix* species, are commonly found in the exoskeleton of wood- and bark-engraver beetles (Klepzig and Six 2004), which are also known to biosynthesize toluene as a byproduct of pheromone production (Gries et al. 1990). Bark beetle infestation has been linked to a 5- to 20-fold increase in the emission of volatile organic compounds, mostly consisting of a wide variety of terpenes and alkylbenzene hydrocarbons (Amin et al. 2012). These results hint to some kind of ecological convergence between fungi growing on alkylbenzenes and aliphatics, as the latter have also been isolated repeatedly from living insects and decaying woody materials (Table 2).

3 Fungal Biotechnology and Hydrocarbons

Hydrocarbonoclastic microorganisms might be both detrimental and beneficial to matters of human interest. On the one hand, the unwanted biodegradation of hydrocarbons occurs in the biodeterioration of fuels and of other materials containing hydrocarbon-related chemicals. These situations require specific biocontrol measures that must be adapted to sometimes very resistant biodeteriogens. On the other hand, the same or related biodegradation processes can also be beneficially used for removing toxic hydrocarbons from contaminated sites and industrial waste streams. As discussed in Sect. 2, the role of fungi in environments that are rich in hydrocarbons and that lack a free water phase – as seen in soil and in solid-state-like bioreactors – cannot be overlooked.

Table 2 Updated identity of conserved fungi that have the capacity to assimilate aliphatic hydrocarbons as sole source of carbon and energy, verified via the measurement of growth curves in axenic cultures, substrate/biomass balances, or by substrate labelling assays

Validated name (Order)	Strain no.[a]	Isolation source (country)	Growth substrates[b]	BSL[c]	References
Amorphotheca resinae (*Leotiomycetes* incertae sedis)	ATCC 22711 ATCC 52833	Kerosene aviation fuel (USA) Naval diesel fuel (UK)	C10-C14, C16 C12	1	(Cofone et al. 1973) (Lindley and Heydeman 1983)
Beauveria bassiana (*Hypocreales*)	IMIZA Bb10	Insect cuticle (AR)	C16	1	(Napolitano and Juárez 1997)
Blastobotrys robertii (*Saccharomycetales*)	CBS 10106	Rotten pine wood (NL)	C16	1	(Middelhoven and Kurtzman 2007)
Candida albicans (*Saccharomycetales*)	KTCC 89062	Soil polluted with crude oil (Kuwait)	C12-C20	1	(Sorkhoh et al. 1990)
Candida boleticola (*Saccharomycetales*)	CBS 10111	Beech whiterot (NL)	C16	1	(Middelhoven 2006)
Candida cretensis (*Saccharomycetales*)	CBS 9453	Rotten mushroom on tamarisk (GR)	C16	1	(Middelhoven and Kurtzman 2007)
Candida keroseneae (*Saccharomycetales*)	IMI 395605	Kerosene aviation fuel (UK)	C16-C18	1	(Buddie et al. 2011)
Candida maltosa (*Saccharomycetales*)	EH 15 EH 60	Unknown Unknown	C12, C16	1	(Chrzanowski et al. 2008)
Candida scorzettiae (*Saccharomycetales*)	CBS 10107	Rotten oak wood (NL)	C16	1	(Middelhoven and Kurtzman 2007)
Candida tropicalis (*Saccharomycetales*)	ATCC 32113	Mud in refinery premises (unknown)	C16	2	(Hug and Fiechter 1972)
Candida vadensis (*Saccharomycetales*)	CBS 9454	Rotten mushroom on beech (NL)	C16	1	(Middelhoven and Kurtzman 2007)

Cryptococcus haglerorum (*Trichosporonales*)	CBS 8902	Nest of leaf cutting ants (BR)	C16	1	(Middelhoven et al. 2003)
Cryptococcus music (*Trichosporonales*)	CBS 9492	Beech brownrot (NL)	C16	1	(Middelhoven 2006)
Metarhizium anisopliae (*Hypocreales*)	IMIZA Ma6	Insect cuticle (AR)	C16	1	(Napolitano and Juárez 1997)
Pichia guilliermondii (*Saccharomycetales*)	H 71	Unknown	C12, C16	1	(Chrzanowski et al. 2008)
Purpureocillium lilacinum (*Hypocreales*)	CBS 284.36	Soil, nematophagous (USA)	C16	1	(Vigueras et al. 2014)
Scedosporium boydii (*Microascales*)	ATCC 58400	Raw sewage (UK)	C2-4	2	(Davies et al. 1973)
Scheffersomyces insectosa (*Saccharomycetales*)	CBS 10110	Beech brownrot (NL)	C16	1	(Middelhoven 2006)
Scheffersomyces ergatensis (*Saccharomycetales*)	CBS 10109	Beech brownrot (NL)	C16	1	(Middelhoven 2006)
Trichosporon guehoae (*Trichosporonales*)	CBS 8521	Pasture soil (NL)	C16	1	(Middelhoven et al. 1999)
Trichosporon veenhuisii (*Trichosporonales*)	CBS 7136	Buffalo dung (IT)	C16	1	(Middelhoven et al. 2000)
Yarrowia lipolytica (*Saccharomycetales*)	ATCC 8662 ATCC 20177	Unknown Kerosene aviation fuel (unknown)	C10, C12, C16	1	(Gutierrez and Erickson 1977) (Sprenger and Rehm 1983)

[a]Strain number equivalents among collections have been verified with StrainInfo (http://www.straininfo.net/). Preferential public collections have been indicated: American Type Culture Collection (ATCC, USA); CBS fungal collection (Fungal Biodiversity Center, NL); collection of the Helmholtz Centre for Environmental Research (UFZ, DE); Laboratorio de Hongos Entomopatógenos (IMYZA-INTA, AR); IMI Fungal collection (International Mycological Institute, UK)

[b]C2: ethane; C3: propane; C4: *n*-butane; 12: *n*-dodecane; C16: *n*-hexadecane; C17: *n*-heptadecane; C18: *n*-octadecane; C20: *n*-icosane

[c]Biosafety level

3.1 Biodeterioration of Oil Derivatives

Fuel spoilage by microorganisms is, despite the generalized practice of blending fuels with biocides, an important economic problem for the petrochemical industry of today (Passman 2013). The accumulation of water at the bottom of storage tanks and in oil pipelines is a primary prerequisite for development of microorganisms. The main concern has been the presence of sulfate-reducing bacteria, which under anoxic conditions use the sulfate in petroleum fuels as the terminal electron acceptor for their electron transport chain. They generate sulfide as waste product, thus greatly contributing to the biocorrosion of carbon steel. Biocontrol efforts targeting these microorganisms have mainly been focused on accurate biomonitoring and on the development of effective, yet environmentally friendly, biocides. Comprehensive reviews have emphasized that the role of fungi in biocorrosion, biofouling, and clogging problems in fuel appliances should not be underestimated (Gaylarde et al. 1999; Little and Ray 2001; Yemashova et al. 2007). The quality of fuel is extremely important in the case of kerosene, which is particularly susceptible to fungal contamination. The engine failure of aircraft using it can have dramatic consequences. A recent survey on the presence of fungi in stored automobile fuels from a tropical environment showed significant colonization at the bottom of the tanks, close to the water interphase. Diesel was generally more contaminated than gasoline (Rodríguez-Rodríguez et al. 2010). Several other petroleum derivatives that are rich in hydrocarbons have also been reported to be affected by fungi: naphtha storage tanks and transporting pipelines (Naranjo et al. 2015), solid and liquid lubricants (technical vaselines, rope and gun oil), cutting fluids, and even bitumens and asphalts consisting of complex mixtures of long-chain aliphatics and PAHs (Yemashova et al. 2007).

In recent years, a relationship between chaetothyrialean black yeasts able to assimilate alkylbenzenes (see Sect. 3.3) and gasoline storage facilities like pump stations and car tanks has been evidenced (Isola et al. 2013; Prenafeta-Boldú et al. 2001a) (Fig. 7). The reason black yeasts have so long been overlooked as potential fuel biodeteriogens may lie in their slow growth and in the low competitive ability of the majority of the species under common laboratory conditions, so that they are easily disregarded in conventional studies. In addition, chaetothyrialean black yeasts are notoriously difficult to identify on morphological grounds and might have been easily confused by other more common melanized fungi (e.g., *Cladosporium sphaerospermum*, *Aureobasidium pullulans*, and even *Amorphotheca resinae*). In fact, it might well be that previous records of the occurrence of the so-called kerosene/creosote fungus *A. resinae* (Sect. 3.1) were actually based on misidentification of chaetothyrialean black yeasts. That would be especially likely for creosoted wood, where black yeasts have appeared as dominant in recent isolation studies (Gümral et al. 2014; Zhao et al. 2010). They tended to be absent in such studies before the general use of molecular phylogeny became established and before specialized isolation methods were applied (David 1954). In support of this idea, results obtained with a novel qPCR protocol for the specific quantification of *A. resinae* have suggested that the incidence of this fungus has been overestimated in

Fig. 7 Black fungal biofilms formed at the inner part of a car fuel tank (left) and internal surface of the lid (right) upon continuous exposure to gasoline vapors. The main strain isolated was identified as the hydrocarbonoclastic species *Exophiala xenobiotica*. (Courtesy of D. Isola)

the past (Martin-Sanchez et al. 2016a). A survey carried out by the same authors on the microbial load in diesel storage tanks using culture- and qPCR-based approaches (Martin-Sanchez et al. 2016b) corroborated the absence of *A. resinae*. Instead, the most prevalent fungi were identified as the well-known hydrocarbonoclastic species *Paecilomyces variotii* and *Scedosporium boydii*, as well as an undescribed yeast belonging to the *Dipodascaceae*.

The extensive adoption of biofuel blends during the twenty-first century has unleashed new challenges related to the preservation of these relatively readily biodegradable mixtures of biological and mineral fuels. Most of the identified fungal contaminants in biodiesel blends have previously been reported in the mineral counterparts (Soriano et al. 2015), but the introduction of vegetable oils has increased the vulnerability of biodiesel to fungal colonization of the bottom water phase with species such as *Paecilomyces variotii* (Gassen et al. 2015). Besides, biodiesel also promotes the biodegradation of the polyethylene films that are commonly used in the manufacture of storage tanks to prevent corrosion (Restrepo-Flórez et al. 2015). This is quite a surprising finding since hydrocarbon-based plastics (polyethylene, polypropylene, and polystyrene) are regarded as highly resistant to microbial attack. Nonetheless, an increasing number of reports have highlighted the importance of bacterial and fungal processes in the deterioration of low-density polyethylene (LDPE) (Restrepo-Flórez et al. 2014). The presence of hydrocarbons in certain matrixes is prompting unforeseen biodeterioration problems. The extraordinary physiological and metabolic adaptability of the black yeasts has yielded additional negative effects as these fungi have been found as emerging biodeteriogens in ancient stone buildings and monumental sites. This phenomenon has been linked to the increasing level of air pollution containing volatile hydrocarbons (Isola et al. 2016), as well as to the application of phenolic biocides (Martin-Sanchez et al. 2012). Recently, black yeasts have been recurrently reported as growing on plastic and rubber surfaces of washing machines and dishwashers, a phenomenon that has raised the issue of biosafety in relation to domestic appliances (Gümral et al. 2016).

3.2 Soil Bioremediation

The term mycoremediation, defined as the use of filamentous fungi for bioremediation purposes, has become increasingly popular in recent years. However, the development of environmental biotechnology using fungi had already been proposed in 1980s for both the *CYP* and the ligninolytic enzymatic systems. Several treatises and reviews have been written since then on fundamental and applied aspects of mycoremediation (Gadd 2001; Kremer and Anke 1997; Purchase 2016). Fungal bioremediation has primarily been considered for pollutant classes and environmental conditions where bacterial biodegradation processes are poorly efficacious. Despite the accumulation of knowledge from numerous laboratory studies, the story of using fungi for the bioremediation of hydrocarbon-polluted sites remains one of mixed success (Harms et al. 2011). Pilot and full-scale projects investigating the bioremediation of PAHs after soil bioaugmentation with white root fungi have indicated that the efficacy of the process may have been limited by the xylicolous nature of the white-rot species used. Suggested bottlenecks in these bioremediation processes have included the inability of the fungi to compete with the native soil microbes, the inadequate handling of nutrient supplements, and use of species in the field under unfavorable environmental conditions of soil moisture, pH, oxygen, or temperature. An additional nutritional factor for some species was the need for supplementing sites with lignocellulosic materials such as sawdust and straw. A significant general limitation in designing field experiments lay in the overall poor understanding and control of enzymatic expression mechanisms in the ligninolytic enzymes (Singleton 2001). Work with white-rot fungi is also affected by the generation of diffusible toxic metabolites, which may inhibit the indigenous microbial populations, preventing the complete biodegradation of PAHs and creating an elevated ecotoxicological risk. Such problems appear to be specific to particular fungal species and target contaminants (Andersson et al. 2003).

These glitches have led to a search for new fungal species with improved bioremediation potential. Litter-decomposing fungi have been suggested as promising candidates in that their physiology tends to be adapted to soil conditions (Pozdnyakova 2012). The biodegradation of PAHs by novel lignin-degrading isolates has been verified in microcosm studies involving an increasing number of common soil-inhabiting ascomycetes (Reyes-César et al. 2014). In general, the biostimulation of indigenous soil populations has been proposed as a more viable strategy than bioaugmentation, provided that a significant microbial biodiversity is already present in the soil. However, regardless of the fungi selected, the bioavailability of hydrocarbon contaminants is a key issue in the success of mycoremediation efforts. Long-term and steady presence of hydrocarbons in the soil matrix ("aged soil pollution") allows time for the migration and sorption of hydrophobic hydrocarbons into relatively inaccessible soil pores, thus rendering these contaminants less available for microbial biodegradation. Several physicochemical treatments, ranging from the in situ surfactant amendment to ex situ solvent extraction, have been tested to improve bioavailability (Singleton 2001). The extra costs, however, must then be balanced with the stringency of the required clean-up.

A relatively inexpensive combined bioagmentation and biostimulation strategy consists of the supplementation of soils with composted materials, in either in situ land-farming approaches or ex situ in biopiles. Besides improving the microbial, nutritional, and structural soil quality parameters, compost has also been found to increase the solubility of certain PAHs (Kobayashi et al. 2009). A study that disputably claimed to be the first field-scale application of a fungal treatment to PAH-contaminated soil showed that, in lab-scale pilot studies, the addition of green compost plus the ligninolytic fungus *Phanerochaete velutina* produced greater biodegradation of high molecular weight PAHs than did the addition of fungally uninoculated compost (Winquist et al. 2014). No significant differences were found between inoculated and uninoculated treatments in the corresponding field-scale experiments. However, a significant increase in numbers of PAH-hydroxylating bacteria was found in the field studies, pointing to the important role of this group of microbes. In any case, apart from bringing about direct biodegradation of hydrocarbons, fungi may have other benefits for bioremediation. Current research is being directed towards synergistic interactions with co-occurring hydrocarbonoclastic bacteria. There have been claims that two different fungal transport mechanisms enhance the bioavailability of pollutants. In one system termed "fungal highways," bacteria use liquid films around fungal hyphae to overcome motility restrictions and to reach hydrocarbon-polluted areas. In the other, denominated as "fungal pipelines," fungi absorb and translocate hydrocarbons through their mycelial networks towards zones containing active bacteria (Banitz et al. 2013).

3.3 Air Biofiltration

Biofiltration has been proposed as a preferred alternative to other physicochemical air pollution control technologies, partly because investment and maintenance costs are seen as relatively low (Ralebitso-Senior et al. 2012). In this type of bioprocess, as stream of gas polluted with volatile organic compounds is passed through a porous support material that offers a large contact area and immobilizes microbial cultures (Fig. 8). In addition to supporting inoculated microbial strains, biofilters are biologically open systems that incorporate cells of a wide variety of microorganisms entering via incident air flow. The absence of a mobile water phase simplifies the reactor configuration and improves the mass transfer of hydrophobic substrates into the active biofilm, where they are biodegraded. However, control of parameters that strongly affect the microbial activity, like pH, water activity, and nutrient supply, is difficult without free-flowing water.

The literature on the purposeful use of fungi in biofilters targeting gaseous contaminants has already been reviewed; most reports strictly concern lab-scale installations (Kennes and Veiga 2004; van Groenestijn et al. 2001). Fungal biofiltration of volatile aliphatics has been investigated recently for *Scedosporium boydii* ATCC 58400, the well-known aliphatic-degrading model strain previously identified as *Graphium* sp. (Sect. 2.1). This fungus played an important role in the degradation of methane in a compost biofilter, but methanol had to be supplemented

Fig. 8 Lab-scale biofilters for the biofiltration of toluene vapors. Columns are packed with polyurethane foam cubes (left) and perlite granules (right) as carrier materials, and inoculated with *Cladophialophora psammophila* CBS110553. A blackish fungal biofilm has grown over the packing materials

as a carbon and energy source to support fungal growth (Lebrero et al. 2016). Longer-chain alkanes like hexane were also efficiently removed in two air biofilters packed with expanded clay and perlite granules and enriched, respectively, with *Aspergillus niger* (Spigno et al. 2003) and with two fungi inexactly identified as *Cladosporium* sp. and *Fusarium* sp. (Arriaga and Revah 2005). An *Ophiostoma* sp. and other unidentified melanized strains were shown to bring about the biodegradation of *a*-pinene in biofilters where this terpene hydrocarbon was treated (Jin et al. 2006; van Groenestijn and Liu 2002).

Fungal biofilters have been studied intensively in the treatment of volatile monoaromatic compounds, including alkylbenzenes such as toluene and styrene (Table 3). This is a logical step considering the known metabolic capacity of certain fungi to assimilate these compounds as growth substrates and to withstand growth-limiting biofilter conditions (see Sect. 2.3). In chaetothyrialean fungi, assimilation of alkylbenzenes appears to be predominant in the genera *Exophiala* and *Cladophialophora*, but the closely related *Fonsecaea* has also been found to become enriched spontaneously in biofilters treating complex mixtures of alkylbenzenes and other volatile compounds (Prenafeta-Boldú et al. 2012; Qi et al. 2005). The fungus *Sporothrix variecibatus*, isolated initially from a biofilter treating styrene vapors, has successfully

Table 3 Updated identity of conserved fungi that have the capacity to assimilate aromatic hydrocarbons and alkylbenzenes as sole source of carbon and energy, verified via the measurement of growth curves in axenic cultures, substrate/biomass balances, or by substrate labelling assays

Validated name (Order)	Strain no.[a]	Isolation source (country)	Growth substrates[b]	BSL[c]	References
Bionectria ochroleuca (*Hypocreales*)	CBS 102.94	Air biofilter degrading styrene (NL)	ETB	1	(Cox et al. 1993a)
Cyphellophora sessilis (*Chaetothyriales*)	CBS 238.93	Air biofilter degrading styrene (NL)	ETB	1	(Cox et al. 1993a)
Cladophialophora exuberans (*Chaetothyriales*)	CRMP 1227 CRMP 1219	Babassu coconuts (BR)	MB	1	(Nascimento et al. 2017)
Cladophialophora immunda (*Chaetothyriales*)	CBS 110551	Soil polluted with gasoline (NL)	MB	1	(Prenafeta-Boldú et al. 2001a)
Cladophialophora psammophila (*Chaetothyriales*)	CBS 110553	Soil polluted with gasoline (NL)	MB, EB, ETB	1	(Prenafeta-Boldú et al. 2001a)
Exophiala lecanii-corni (*Chaetothyriales*)	CBS 102400	Air biofilter degrading toluene (US)	MB	2	(Woertz et al. 2001)
Exophiala mesophila (*Chaetothyriales*)	CBS 120910	Human host (US)	MB	2	(Blasi et al. 2016)
Exophiala oligosperma (*Chaetothyriales*)	CBS 814.95 CBS 113408	Air biofilter degrading styrene (NL) Air biofilter degrading toluene (SP)	ETB MB	2	(Cox et al. 1993b) (Estevez et al. 2005)
Exophiala xenobiotica (*Chaetothyriales*)	CBS 110555	Soil polluted with gasoline (DE)	MB	2	(Prenafeta-Boldú et al. 2001a)
Fusarium solani (*Hypocreales*)	UCEIV S19	Compost contaminated with oil (FR)	BaP	2	(Rafin et al. 2000)
Polycephalomyces sinensis (*Hypocreales*)	CBS 113409 CBS 115145	Air biofilter degrading toluene (SP) Air biofilter degrading toluene (MX)	MB MB	1	(Estevez et al. 2005) (García-Peña et al. 2008)
Sporothrix schenckii (*Ophiostomatales*)	CBS 110552	Air biofilter treating toluene (NL)	MB	2	(Prenafeta-Boldú et al. 2001a)

[a]Strain number equivalents among collections have been verified with StrainInfo (http://www.straininfo.net/). Preferential public collections have been indicated: CBS Fungal Collection (Fungal Biodiversity Center, NL); UCEIV Mycology Collection (Unité de Chimie Environnementale et Interactions sur le Vivant, FR); CRM-UNESP Fungal Collection (Central de Recursos Microbianos do Instituto de Biociências – UNESP, BR)

[b]MB: toluene; EB: ethylbenzene; ETB: styrene; BaP: benzo[*a*]pyrene

[c]Biosafety level

been applied in biofilter experiments in a variety of mixtures and in various operational conditions (Rene et al. 2010). Unfortunately, that strain was not preserved (personal communication). The relatively high efficiency levels obtained in these studies have been attributed to the hydrophobic nature and extensive surface-contact area of aerial hyphae. Co-occurring bacteria still played a fundamental role in systems where poorly biodegradable short-chain alkanes (Halecky et al. 2015) and benzene (Prenafeta-Boldú et al. 2012) were present. This was also true in complex mixtures of several volatile compounds whose collective degradation required a high metabolic diversity among the microbial consortium.

3.4 Biosafety Issues

The metabolic capacity to assimilate alkylbenzenes in the *Chaetothyriales* is also associated with a tendency to cause severe infections in humans, with a preference towards the central nervous system in certain species (Prenafeta-Boldú et al. 2006). *Cladophialophora bantiana*, a pathogenic BSL3 species associated almost exclusively with severe brain infections, is perhaps the most representative example of this trend. It has been speculated that such dual ecophysiological traits might converge in the mammal brain, because of its particular chemistry rich in monoaromatic neurotransmitters and neuromelanin components. Recent findings suggest that chaetothyrialean fungi are undergoing a process of strong evolutionary radiation. Not uncommonly, clades feature the occurrence of closely related sibling species, some of whose members are evolving towards virulence or towards the metabolism of alkylbenzenes and related monoaromatic toxic pollutants (Kaltseis et al. 2009; Teixeira et al. 2017). This phenomenon is illustrated by the toluene-assimilating *Cladophialophora psammophila* which, despite being phylogenetically very similar to *C. bantiana*, appears to be completely avirulent (Badali et al. 2011). However, precise delimitation between hydrocarbonoclastic and pathogenic species appears to be dubious in certain cases, as with the toluene-growing *Exophiala oligosperma*, *E. xenobiotica*, and *E. mesophila*, which are recognized as BSL2 human pathogens commonly associated to opportunistic infections (Table 3). The genome of the closely related *Rhinocladiella mackenziei*, another neurotropic BSL3 species endemic from Saudi Arabia and Pakistan, encompasses several of the genes related to the assimilation pathway of toluene (Moreno et al. 2018). Despite inhabiting an oil-rich biogeographical area, confirmatory cultivation with this substrate has never been attempted. Significant progress is expected on the understanding of the interrelation between these apparently poorly unrelated ecological traits thanks to the full genome sequencing of a number of hydrocarbonoclastic and pathogenic black yeasts (Teixeira et al. 2017).

4 Research Needs

Understanding the biodegradation of hydrocarbons by fungi involve studying multiple aspects that are related to basic genetic, evolutionary, and ecological functions of hydrocarbonoclastic metabolism. Important advancements have been achieved in recent years, but there are some major scientific challenges that need to be addressed. Concerning the applied aspects, there is still room for innovation in rather mature technological processes, like bioremediation and biofiltration of hydrocarbon pollution, but the gained fundamental knowledge might spur further research on emerging biotechnological concepts. In summary, the following research topics are proposed:

- *Ecophysiology and evolution of the fungal metabolism of hydrocarbons*: While coincidental metabolism of hydrocarbons in fungi appears to be related to the detoxification of xenobiotics and the biodegradation of lignin, the assimilatory metabolism might have evolved from complex fungal-plant-insect biotrophic interactions. Hydrocarbons form a constituent part of the insect's cuticle and also act as volatile signaling compounds for both insects and plants. As yet, these habitats have not been systematically investigated concerning this hypothesis. Comprehensive isolation programs in environments like the galleries of bark-boring beetles and the nests of leaf-cutting ants might broaden our views on the biodiversity and ecology of fungi growing on hydrocarbons. These studies should employ a combination of specialized enrichment and isolation techniques and culture-independent molecular methods. The obtained results must then be analyzed from the perspective of basic ecological and evolutionary principles for a better understanding of the fungal assimilation of hydrocarbons.
- *Interactions between fungal hydrocarbonoclastic enzymes*: When compared to bacterial enzymes systems, fungal enzymes responsible for the break-down of hydrocarbons are remarkably diverse. This complexity might have prompted a certain reductionism among researchers in their approach to understanding the specific biochemical reactions, leading to each individual process being conceived as independent of the others. The recent observation that strains from rather distantly related hydrocarbonoclastic species, such as *Fusarium solani* and *Phanerochaete chrysosporium*, simultaneously express many of the major hydrocarbonoclastic enzyme groups, including *CYP* monooxygenases, peroxidases, and laccases, highlights the importance of studying the synergistic interactions among these enzyme systems. Genomic and transcriptomic studies will shed new insights into the intrinsic and extrinsic regulation and expression of these enzymes. Practical applications may ensue.
- *Fungal community structure and function in biologically contaminated fuels*: Despite the advancement and broad use of high throughput molecular tools, these techniques have scarcely been applied for understanding the biodiversity of fuel biodeteriogens. Regular implementation of culture independent microbiome approaches in the study of samples taken from biologically contaminated fuel tanks will help to clarify the identity, role, and importance of emerging fungal

biodeteriogens. These studies will also clarify the status of potentially overemphasized organisms like *Amorphotheca resinae*, the relevance of which has recently been disputed. A precise identification of hydrocarbonoclastic fungi from environmental samples based upon molecular phylogeny and detection tools will facilitate the implementation of a more effective bioprotection of hydrocarbon materials. The biosafety of biotechnological solutions will also be improved.

- *Fungal assimilatory biodegradation of polycyclic hydrocarbons*: The complete biodegradation of hydrocarbon contaminants to carbon dioxide and water is the most desirable process considering that, in incomplete breakdown processes, some of the materials produced might be more toxic than the parent substrates. Despite the breakthrough that some rather ubiquitous fungi were found to be able to grow on the highly recalcitrant priority toxic pollutant benzo[*a*]pyrene as the sole source of carbon and energy, relatively little is known about the intra- and interspecific biodiversity of fungal species with this trait, or about the metabolic routes involved. Complete biodegradation of hydrocarbons can also be achieved through synergistic interactions between fungi and bacteria, and the fungal/bacterial microbiome approach should be widely applied in soil bioremediation air biofiltration studies, in order to link microbial community structure and dynamics to process efficiency.
- *Extremophilic hydrocarbonoclastic fungi as source of new industrial enzymes*: The strongly melanized black yeasts occur in a wide range of extreme environments: high and low temperatures and pH, dry and salty conditions, exposure to ionizing radiation, etc. Such evolutionarily driven adaptability may be reflected in diverse enzymatic processes that should be equally adapted to withstand extreme conditions. These so-called extremozymes are of great interest in industrial and environmental biotechnology as long-life biocatalytic agents that can be combined with chemical processes. Examples include paper biopulping and biobleaching, textile treatments, and detoxification of phenolic wastes. Laccases, for example, are being investigated in emerging applications, such as catalysts for the manufacture of polymers and even anticancer drugs, but also in the development of biosensors and nanobiotechnology concepts.
- *Biosafety of fungal biotechnological applications*: Phylogenetically unrelated fungi from the *Microascales* and the *Chaetothyriales* have the particularity of having two significant ecological functions that affect humans: they are hydrocarbonoclastic fungi in natural and anthropized environments but are also important human opportunistic pathogens. In addition, these fungi are also characterized by an extremophilic ecology, which makes them very suitable as biocatalysts for the biodegradation of hydrocarbons in soils (bioremediation) and solid state-like bioreactors (biofilters). Therefore, precise phylogenetic delimitation and characterization of pathogenic species is fundamental to preventing the generation of biohazard in polluted materials. This is particularly crucial in biofiltration of air streams, considering the risk of aerosolization of fungal spores.

Acknowledgments This research was supported by the CERCA Programme/Generalitat de Catalunya and by the IRTA Seed Research Fund Program. Francesc Prenafeta-Boldú is the coordinator of the Consolidated Research Group TERRA, funded by the Generalitat de Catalunya (2017 SGR

1290). We are thankful to Leandro Moreno for the critical reading of the manuscript. The collaboration of Daniela Isola, Derlene Attili de Angelis, and Fernando Carlos Pagnocca in providing graphical material is also acknowledged.

References

Amin H, Atkins PT, Russo RS, Brown AW, Sive B, Hallar AG, Huff Hartz KE (2012) Effect of bark beetle infestation on secondary organic aerosol precursor emissions. Environ Sci Technol 46(11):5696–5703

Andersson BE, Lundstedt S, Tornberg K, Schnürer Y, Öberg LG, Mattiasson B (2003) Incomplete degradation of polycyclic aromatic hydrocarbons in soil inoculated with wood-rotting fungi and their effect on the indigenous soil bacteria. Environm Toxicol Chem 22(6):1238–1243

April TM, Abbott SP, Foght JM, Currah RS (1998) Degradation of hydrocarbons in crude oil by the ascomycete *Pseudallescheria boydii* (*Microascaceae*). Can J Microbiol 44(3):270–278

Aranda E, Marco-Urrea E, Caminal G, Arias ME, García-Romera I, Guillén F (2010) Advanced oxidation of benzene, toluene, ethylbenzene and xylene isomers (BTEX) by *Trametes versicolor*. J Hazard Mater 181(1):181–186

Arriaga S, Revah S (2005) Improving hexane removal by enhancing fungal development in a microbial consortium biofilter. Biotechnol Bioeng 90(1):107–115

Asha S, Vidyavathi M (2009) *Cunninghamella* – a microbial model for drug metabolism studies – a review. Biotechnol Adv 27(1):16–29

Badali H, Gueidan C, Najafzadeh MJ, Bonifaz A, van den Ende AHGG, de Hoog GS (2008) Biodiversity of the genus *Cladophialophora*. Stud Mycol 61(1):175–191

Badali H, Carvalho VO, Vicente VA, Attili-Angelis D, Kwiatkowski IB, Van den Ende AHG, de Hoog GS (2009) *Cladophialophora saturnica* sp. nov., a new opportunistic species of *Chaetothyriales* revealed using molecular data. Med Mycol 47(1):51–62

Badali H, Prenafeta-Boldú FX, Guarro J, Klaassen C, Meis JF, de Hoog GS (2011) *Cladophialophora psammophila*, a novel species of *Chaetothyriales* with a potential use in the bioremediation of volatile aromatic hydrocarbons. Fungal Biol 115(10):1019–1029

Baldrian P (2006) Fungal laccases – occurrence and properties. FEMS Microbiol Rev 30(2): 215–242

Banitz T, Johst K, Wick LY, Schamfuß S, Harms H, Frank K (2013) Highways versus pipelines: contributions of two fungal transport mechanisms to efficient bioremediation. Environ Microbiol Rep 5(2):211–218

Barth G, Gaillardin C (1996) Nonconventional yeasts in biotechnology: a handbook. Springer, Berlin/Heidelberg, pp 313–388

Beopoulos A, Desfougeres T, Sabirova J, Zinjarde S, Neuvéglise C, Nicaud J-M (2010) In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin/Heidelberg, pp 2111–2121

Blasi B, Poyntner C, Rudavsky T, Prenafeta-Boldú FX, Hoog SD, Tafer H, Sterflinger K (2016) Pathogenic yet environmentally friendly? Black fungal candidates for bioremediation of pollutants. Geomicrobiol J 33(3–4):308–317

Blasi B, Tafer H, Kustor C, Poyntner C, Lopandic K, Sterflinger K (2017) Genomic and transcriptomic analysis of the toluene degrading black yeast *Cladophialophora* immunda. Sci Rep 7(1):11436

Boulton C, Ratledge C (1984) Physiology of hydrocarbon-utilizing microorganisms. Top Enzym Ferment Biotechnol 9:11–77

Buddie AG, Bridge PD, Kelley J, Ryan MJ (2011) *Candida keroseneae* sp. nov., a novel contaminant of aviation kerosene. Lett Appl Microbiol 52(1):70–75

Cañero DC, Roncero MIG (2008) Functional analyses of laccase genes from *Fusarium oxysporum*. Phytopathology 98(5):509–518

Cerniglia CE, Sutherland JB (2010) In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin/Heidelberg, pp 2079–2110

Cerniglia CE, Sutherland JB, Crow SA (1992) In: Winkelmann G (ed) Microbial degradation of natural products. VCH Verlagsgesellschaft, Weinheim, pp 193–217

Chen W, Lee M-K, Jefcoate C, Kim S-C, Chen F, Yu J-H (2014) Fungal cytochrome P450 monooxygenases: their distribution, structure, functions, family expansion, and evolutionary origin. Genome Biol Evol 6(7):1620–1634

Chrzanowski Ł, Bielicka-Daszkiewicz K, Owsianiak M, Aurich A, Kaczorek E, Olszanowski A (2008) Phenol and *n*-alkanes (C12 and C16) utilization: influence on yeast cell surface hydrophobicity. World J Microbiol Biotechnol 24(9):1943–1949

Cofone L, Walker JD, Cooney JJ (1973) Utilization of hydrocarbons by *Cladosporium resinae*. Microbiology 76(1):243–246

Cox HHJ, Houtman JHM, Doddema HJ, Harder W (1993a) Enrichment of fungi and degradation of styrene in biofilters. Biotechnol Lett 15(7):737–742

Cox HHJ, Houtman JHM, Doddema HJ, Harder W (1993b) Growth of the black yeast *Exophiala jeanselmei* on styrene and styrene-related compounds. Appl Microbiol Biotechnol 39(3): 372–376

Cox HHJ, Faber BW, Van Heiningen WNM, Radhoe H, Doddema HJ, Harder W (1996) Styrene metabolism in *Exophiala jeanselmei* and involvement of a cytochrome P-450-dependent styrene monooxygenase. Appl Environ Microbiol 62(4):1471–1474

Curry S, Ciuffetti L, Hyman M (1996) Inhibition of growth of a *Graphium* sp. on gaseous *n*-alkanes by gaseous *n*-alkynes and *n*-alkenes. Appl Environ Microbiol 62(6):2198–2200

Dallinger A, Duldhardt I, Kabisch J, Schlüter R, Schauer F (2016) Biotransformation of cyclohexane and related alicyclic hydrocarbons by *Candida maltosa* and *Trichosporon* species. Int Biodeter Biodegr 107:132–139

David HM (1954) Studies of the creosote fungus, *Hormodendrum resinae*. Mycologia 46(2): 161–183

Davies JS, Wellman AM, Zajic JE (1973) Hyphomycetes utilizing natural gas. Can J Microbiol 19(1):81–85

de Boer W, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29(4):795–811

de Hoog GS (1999) Ecology and evolution of black yeasts and their relatives. Stud Mycol 43:1–208

de Hoog GS, Vicente VA, Caligiorne RB, Kantarcioglu S, Tintelnot K, Gerrits van den Ende AHG, Haase G (2003) Species diversity and polymorphism in the *Exophiala spinifera* clade containing opportunistic black yeast-like fungi. J Clin Microbiol 41(10):4767–4778

Duarte APM, Attili-Angelis D, Baron NC, Forti LC, Pagnocca FC (2014) Leaf-cutting ants: an unexpected microenvironment holding human opportunistic black fungi. Antonie Van Leeuwenhoek 106(3):465–473

Estevez E, Veiga MC, Kennes C (2005) Biodegradation of toluene by the new fungal isolates *Paecilomyces variotii* and *Exophiala oligosperma*. J Ind Microbiol Biotechnol 32(1):33–37

Fedorak PM, Westlake DWS (1986) Fungal metabolism of *n*-alkylbenzenes. Appl Environ Microbiol 51(2):435–437

Fickers P, Benetti PH, Waché Y, Marty A, Mauersberger S, Smit MS, Nicaud JM (2005) Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. FEMS Yeast Res 5(6–7):527–543

Gadd GM (2001) Fungi in bioremediation. Cambridge University Press, Cambridge

García-Peña I, Ortiz I, Hernández S, Revah S (2008) Biofiltration of BTEX by the fungus *Paecilomyces variotii*. Int Biodeter Biodegr 62(4):442

Gassen J, Bento F, Frazzon A, Ferrão M, Marroni I, Simonetti A (2015) Growth of *Paecilomyces variotii* in B0 (diesel), B100 (biodiesel) and B7 (blend), degradation and molecular detection. Braz J Biol 75:541–547

Gaylarde CC, Bento FM, Kelley J (1999) Microbial contamination of stored hydrocarbon fuels and its control. Rev Microbiol 30(1):1–10

Ghosal D, Ghosh S, Dutta TK, Ahn Y (2016) Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Front Microbiol 7:1369
Gibson DT, Subramanian V (1984) In: Gibson DT (ed) Microbial degradation of organic compounds. Marcel Dekker, New York, pp 181–252
Gold MH, Alic M (1993) Molecular-biology of the lignin-degrading basidiomycete phanerochaete-chrysosporium. Microbiol Rev 57(3):605–622
Gries G, Smirle MJ, Leufven A, Miller DR, Borden JH, Whitney HS (1990) Conversion of phenylalanine to toluene and 2-phenylethanol by the pine engraver *Ips-pini* (Say) (*Coleoptera, Scolytidae*). Experientia 46(3):329–331
Gümral R, Tümgör A, Saraçlı MA, Yıldıran ŞT, Ilkit M, de Hoog GS (2014) Black yeast diversity on creosoted railway sleepers changes with ambient climatic conditions. Microb Ecol 68(4):699–707
Gümral R, Özhak-Baysan B, Tümgör A, Saraçlı MA, Yıldıran ŞT, Ilkit M, Zupančič J, Novak-Babič M, Gunde-Cimerman N, Zalar P, de Hoog GS (2016) Dishwashers provide a selective extreme environment for human-opportunistic yeast-like fungi. Fungal Divers 76(1):1–9
Gutierrez JR, Erickson LE (1977) Hydrocarbon uptake in hydrocarbon fermentations. Biotechnol Bioeng 19(9):1331–1349
Halecky M, Rousova J, Paca J, Kozliak E, Seames W, Jones K (2015) Biofiltration of gasoline and diesel aliphatic hydrocarbons. J Air Waste Manage Assoc 65(2):133–144
Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169(1):1–15
Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9(3):177–192
Hasegawa Y, Yoshioka N, Obata H, Kawate S, Yoshizako F, Kaneda T, Tokuyama T (1990) Degradation of cyclohexanone by *Exophiala jeanselmei*. Nippon Nogeikagaku Kaishi 64(2):157–162
Heider J, Schühle K (2013) In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes: prokaryotic physiology and biochemistry. Springer, Berlin/Heidelberg, pp 605–634
Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzym Microb Technol 30(4):454–466
Hölker U, Bend J, Pracht R, Tetsch L, Muller T, Hofer M, de Hoog GS (2004) *Hortaea acidophila*, a new acid-tolerant black yeast from lignite. Antonie Van Leeuwenhoek 86(4):287–294
Hug H, Fiechter A (1972) Assimilation of aliphatic hydrocarbons by *Candida tropicalis*. Arch Mikrobiol 88(2):77–86
Ishijima SA, Yamada T, Maruyama N, Abe S (2017) *Candida albicans* adheres to chitin by recognizing *N*-acetylglucosamine (GlcNAc). Med Mycol J 58(1):E15–E21
Isola D, Selbmann L, Hoog GS, Fenice M, Onofri S, Prenafeta-Boldú F, Zucconi L (2013) Isolation and screening of black fungi as degraders of volatile aromatic hydrocarbons. Mycopathologia 175(5–6):369–379
Isola D, Zucconi L, Onofri S, Caneva G, de Hoog GS, Selbmann L (2016) Extremotolerant rock inhabiting black fungi from Italian monumental sites. Fungal Divers 76(1):75–96
Janda-Ulfig K, Ulfig K, Cano J, Guarro J (2008) A study of the growth of *Pseudallescheria boydii* isolates from sewage sludge and clinical sources on tributyrin, rapeseed oil, biodiesel oil and diesel. Ann Agric Environ Med 15(1):45–49
Jin Y, Veiga MC, Kennes C (2006) Performance optimization of the fungal biodegradation of α-pinene in gas-phase biofilter. Process Biochem 41(8):1722
Juhasz AL, Naidu R (2000) Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[*a*]pyrene. Int Biodeter Biodegr 45(1):57–88
Kadri T, Rouissi T, Kaur Brar S, Cledon M, Sarma S, Verma M (2017) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: a review. J Environ Sci 51:52–74
Kaltseis J, Rainer J, De Hoog GS, Kaltseis J, Rainer J, De Hoog GS (2009) Ecology of *Pseudallescheria* and *Scedosporium* species in human-dominated and natural environments and their distribution in clinical samples. Med Mycol 47(4):398–405

Kennes C, Veiga MC (2004) Fungal biocatalysts in the biofiltration of VOC-polluted air. J Biotechnol 113(1–3):305–319
Kennes C, Veiga MC (2012) In: Singh SN (ed) Microbial degradation of xenobiotics. Springer, Berlin/Heidelberg, pp 177–188
Klepzig KD, Six DL (2004) Bark beetle-fungal symbiosis: context dependency in complex associations. Symbiosis 37(1–3):189–205
Kobayashi T, Murai Y, Tatsumi K, Iimura Y (2009) Biodegradation of polycyclic aromatic hydrocarbons by *Sphingomonas* sp. enhanced by water-extractable organic matter from manure compost. Sci Total Environ 407(22):5805–5810
Kohlmeier S, Smits THM, Ford RM, Keel C, Harms H, Wick LY (2005) Taking the fungal highway: mobilization of pollutant-degrading bacteria by fungi. Environ Sci Technol 39(12):4640–4646
Kremer S, Anke H (1997) In: Anke T (ed) Fungal biotechnology. Chapman & Hall, Weinheim, pp 275–295
Lamb DC, Lei L, Warrilow AGS, Lepesheva GI, Mullins JGL, Waterman MR, Kelly SL (2009) The first virally encoded cytochrome P450. J Virol 83(16):8266–8269
Lebrero R, López JC, Lehtinen I, Pérez R, Quijano G, Muñoz R (2016) Exploring the potential of fungi for methane abatement: performance evaluation of a fungal-bacterial biofilter. Chemosphere 144:97–106
Leelaruji W, Buathong P, Kanngan P, Piamtongkam R, Chulalaksananukul S, Wattayakorn G, Chulalaksananukul W (2014) Potential of laccase produced from microfungus, *Aureobasidium pullulans* var. *melanogenum*, to degrade poly-aromatic hydrocarbons. Eur Chem Bull 3(3):269–272
Lindley ND (1992) In: Arora DK, Elander RP, Mukerji KG (eds) Handbook of applied mycology. Marcel Dekker, New York, pp 905–929
Lindley ND, Heydeman MT (1983) Uptake of vapour phase [14C]dodecane by whole mycelia of *Cladosporium resinae*. Microbiology 129(7):2301–2305
Little B, Ray R (2001) A review of fungal influenced corrosion. Corros Rev 19(5–6):401
Luykx DMA, Prenafeta-Boldú FX, de Bont JAM (2003) Toluene monooxygenase from the fungus *Cladosporium sphaerospermum*. Biochem Biophys Res Commun 312:373–379
Majcherczyk A, Johannes C, Hüttermann A (1998) Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of *Trametes versicolor*. Enzym Microb Technol 22(5):335–341
Marco-Urrea E, García-Romera I, Aranda E (2015) Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. New Biotechnol 32(6):620–628
Martin-Sanchez PM, Nováková¡ A, Bastian F, Alabouvette C, Saiz-Jimenez C (2012) Use of biocides for the control of fungal outbreaks in subterranean environments: the case of the Lascaux Cave in France. Environ Sci Technol 46(7):3762–3770
Martin-Sanchez PM, Gorbushina AA, Kunte H-J, Toepel J (2016a) A novel qPCR protocol for the specific detection and quantification of the fuel-deteriorating fungus *Hormoconis resinae*. Biofouling 32(6):635–644
Martin-Sanchez PM, Gorbushina AA, Toepel J (2016b) Quantification of microbial load in diesel storage tanks using culture- and qPCR-based approaches. Int Biodeter Biodegr 126:216–223
Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60(6):551–565
Middelhoven WJ (2006) Polysaccharides and phenolic compounds as substrate for yeasts isolated from rotten wood and description of *Cryptococcus fagi* sp.nov. Antonie Van Leeuwenhoek 90(1):57–67
Middelhoven WJ, Kurtzman CP (2007) Four novel yeasts from decaying organic matter: *Blastobotrys robertii* sp. nov., *Candida cretensis* sp. nov., *Candida scorzettiae* sp. nov. and *Candida vadensis* sp. nov. Antonie Van Leeuwenhoek 92(2):233–244
Middelhoven WJ, Scorzetti G, Fell JW (1999) *Trichosporon guehoae* sp.nov., an anamorphic basidiomycetous yeast. Can J Microbiol 45(8):686–690
Middelhoven WJ, Scorzetti G, Fell JW (2000) *Trichosporon veenhuisii* sp. nov., an alkane-assimilating anamorphic basidiomycetous yeast. Int J Syst Evol Microbiol 50(1):381–387

Middelhoven WJ, Fonseca A, Carreiro SC, Carlos Pagnocca F, Bueno OC (2003) *Cryptococcus haglerorum*, sp. nov., an anamorphic basidiomycetous yeast isolated from nests of the leaf-cutting ant *Atta sexdens*. Antonie Van Leeuwenhoek 83(2):167–174

Moreno LF, Feng P, Weiss VA, Vicente VA, Stielow JB, de Hoog S (2017) Phylogenomic analyses reveal the diversity of laccase-coding genes in *Fonsecaea genomes*. PLoS One 12(2):e0171291

Moreno LF, Ahmed AAO, Brankovics B, Cuomo CA, Menken SBJ, Taj-Aldeen SJ, Faidah H, Stielow JB, de M Teixeira M, Prenafeta-Boldú FX, Vicente VA, de Hoog S (2018) Genomic understanding of an infectious brain disease from the desert. G3 (in press), 8, 300421

Mulheirn L, Van Eyk J (1981) Microbiological oxidation of steroid hydrocarbons. Microbiology 126(2):267–275

Muncnerova D, Augustin J (1994) Fungal metabolism and detoxification of polycyclic aromatic hydrocarbons: a review. Bioresour Technol 48(2):97–106

Napolitano R, Juárez MP (1997) Entomopathogenous fungi degrade epicuticular hydrocarbons of *Triatoma infestans*. Arch Biochem Biophys 344(1):208–214

Naranjo L, Pernía B, Inojosa Y, Rojas D, D'Anna LS, González M, Sisto ÁD (2015) First evidence of fungal strains isolated and identified from naphtha storage tanks and transporting pipelines in Venezuelan oil facilities. Adv Microbiol 05(02):12

Nascimento MMF, Vicente VA, Bittencourt JVM, Gelinski JML, Prenafeta-Boldú FX, Romero-Güiza M, Fornari G, Gomes RR, Santos GD, Gerrits Van Den Ende AHG, de Azevedo CDMPS, De Hoog GS (2017) Diversity of opportunistic black fungi on babassu coconut shells, a rich source of esters and hydrocarbons. Fungal Biol 121(5):488–500

Onodera M, Sakai H, Endo Y, Ogasawara N (1990) Oxidation of short-chain isoalkanes by gaseous hydrocarbon assimilating mold, *Scedosporium* sp. A-4. Agric Biol Chem 53(7):1947–1989

Osono T (2007) Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecol Res 22(6):955–974

Parbery D (1971) Biological problems in jet aviation fuel and the biology of *Amorphotheca resinae*. Mater Org 6:161–208

Passman FJ (2013) Microbial contamination and its control in fuels and fuel systems since 1980 – a review. Int Biodeter Biodegr 81:88–104

Potin O, Veignie E, Rafin C (2004) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by *Cladosporium sphaerospermum* isolated from an aged PAH contaminated soil. FEMS Microbiol Ecol 51(1):71–78

Pozdnyakova NN (2012) Involvement of the ligninolytic system of white-rot and litter-decomposing fungi in the degradation of polycyclic aromatic hydrocarbons. Biotechnol Res Int 2012:20

Prenafeta-Boldú FX, Kuhn A, Luykx D, Anke H, van Groenestijn JW, de Bont JAM (2001a) Isolation and characterisation of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. Mycol Res 105(4):477–484

Prenafeta-Boldú FX, Luykx DMA, Vervoort J, de Bont JAM (2001b) Fungal metabolism of toluene: monitoring of fluorinated analogs by ^{19}F nuclear magnetic resonance spectroscopy. Appl Environ Microbiol 67(3):1030–1034

Prenafeta-Boldú FX, Vervoort J, Grotenhuis JTC, van Groenestijn JW (2002) Substrate interactions during the biodegradation of benzene, toluene, ethylbenzene, and xylene (BTEX) hydrocarbons by the fungus *Cladophialophora* sp. strain T1. Appl Environ Microbiol 68(6):2660–2665

Prenafeta-Boldú FX, Summerbell R, de Hoog GS (2006) Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? FEMS Microbiol Rev 30:109–130

Prenafeta-Boldú FX, Guivernau M, Gallastegui G, Viñas M, de Hoog GS, Elías A (2012) Fungal/bacterial interactions during the biodegradation of TEX hydrocarbons (toluene, ethylbenzene and *p*-xylene) in gas biofilters operated under xerophilic conditions. FEMS Microbiol Ecol 80(3):722–734

Prince RC (2010) In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin/Heidelberg, pp 2065–2078

Purchase D (2016) Fungal applications in sustainable environmental biotechnology. Springer, Cham

Qi B, Moe W, Kinney K (2002) Biodegradation of volatile organic compounds by five fungal species. Appl Microbiol Biotechnol 58(5):684–689

Qi B, Moe WM, Kinney KA (2005) Treatment of paint spray booth off-gases in a fungal biofilter. J Environ Eng ASCE 131(2):180–189

Rafin C, Potin O, Veignie E, Sancholle M (2000) Degradation of benzo[*a*]pyrene as sole carbon source by a non white rot fungus, *Fusarium solani*. Polycycl Aromat Compd 21(1):311–329

Rafin C, Veignie E, Woisel P, Cazier F, Surpateanu G (2008) Modern multidisciplinary applied microbiology. Wiley-VCH, Weinheim, pp 546–550

Raj HG, Saxena M, Allameh A (1992) In: Arora DK, Elander RP, Mukerji KG (eds) Handbook of applied mycology. Marcel Dekker, New York, pp 881–904

Ralebitso-Senior TK, Senior E, Di Felice R, Jarvis K (2012) Waste gas biofiltration: advances and limitations of current approaches in microbiology. Environ Sci Technol 46(16):8542–8573

Reddy CA, D'Souza TM (1994) Physiology and molecular biology of the lignin peroxidases of *Phanerochaete chrysosporium*. FEMS Microbiol Rev 13:137–152

Rehm HJ, Reiff J (1981) In: Fiechter A (ed) Advances in biochemical engineering. Springer, Berlin, pp 175–215

Rene ER, Veiga MC, Kennes C (2010) Biodegradation of gas-phase styrene using the fungus *Sporothrix variecibatus*: impact of pollutant load and transient operation. Chemosphere 79(2):221–227

Restrepo-Flórez J-M, Bassi A, Thompson MR (2014) Microbial degradation and deterioration of polyethylene – a review. Int Biodeter Biodegr 88:83–90

Restrepo-Flórez J-M, Wood JA, Rehmann L, Thompson M, Bassi A (2015) Effect of biodiesel on biofilm biodeterioration of linear low density polyethylene in a simulated fuel storage tank. J Energy Resour Technol 137(3):032211-032211-032216

Reyes-César A, Absalón ÁE, Fernández FJ, González JM, Cortés-Espinosa DV (2014) Biodegradation of a mixture of PAHs by non-ligninolytic fungal strains isolated from crude oil-contaminated soil. World J Microbiol Biotechnol 30(3):999–1009

Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, De la Fuente G, Falcon MA (1996) Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti. FEMS Microbiol Ecol 21(3):213–219

Rodríguez-Rodríguez CE, Rodríguez E, Blanco R, Cordero I, Segura D (2010) Fungal contamination of stored automobile-fuels in a tropical environment. J Environ Sci 22(10):1595–1601

Rosenberg E (2013) In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes: prokaryotic physiology and biochemistry. Springer, Berlin/Heidelberg, pp 201–214

Ruiz-Dueñas FJ, Morales M, García E, Miki Y, Martínez MJ, Martínez AT (2009) Substrate oxidation sites in versatile peroxidase and other basidiomycete peroxidases. J Exp Bot 60(2):441–452

Saparrat MCN, Martínez MJ, Tournier HA, Cabello MN, Arambarri AM (2000) Production of ligninolytic enzymes by *Fusarium solani* strains isolated from different substrata. World J Microbiol Biotechnol 16(8):799–803

Sariaslani FS (1991) Microbial cytochromes P-450 and xenobiotic metabolism. Adv Appl Microbiol 36:133–178

Satow MM, Attili-Angelis D, de Hoog GS, Angelis DF, Vicente VA (2008) Selective factors involved in oil flotation isolation of black yeasts from the environment. Stud Mycol 61(1):157–163

Sikkema J, de Bont J, Poolman B (1995) Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59(2):201–222

Singleton I (2001) In: Gadd G (ed) Fungi in bioremediation. Cambridge University Press, Cambridge, pp 79–96

Soriano AU, Martins LF, Santos de Assumpção Ventura E, Teixeira Gerken de Landa FH, de Araújo Valoni É, Dutra Faria FR, Ferreira RF, Kremer Faller MC, Valério RR, Catharine de Assis Leite D, Lima do Carmo F, Peixoto RS (2015) Microbiological aspects of biodiesel and biodiesel/diesel blends biodeterioration. Int Biodeter Biodegr 99:102–114

Sorkhoh NA, Ghannoum MA, Ibrahim AS, Stretton RJ, Radwan SS (1990) Growth of *Candida albicans* on hydrocarbons: influence on lipids and sterols. Microbios 64:260–261

Spigno G, Pagella C, Fumi MD, Molteni R, de Faveri DM (2003) VOCs removal from waste gases: gas-phase bioreactor for the abatement of hexane by *Aspergillus niger*. Chem Eng Sci 58(3–6):739–746

Sprenger B, Rehm HJ (1983) Biomass production by *Candida* species from *n*-alkanes in a film-submerged reactor in comparison with known culture methods. Eur J Appl Microbiol Biotechnol 17(1):64–68

Steffen KT, Schubert S, Tuomela M, Hatakka A, Hofrichter M (2007) Enhancement of bioconversion of high-molecular mass polycyclic aromatic hydrocarbons in contaminated non-sterile soil by litter-decomposing fungi. Biodegradation 18(3):359–369

Sutherland J (2003) Fungal biotechnology in agricultural, food, and environmental applications. CRC Press, New York, pp 443–456

Syed K, Porollo A, Lam YW, Grimmett PE, Yadav JS (2013) CYP63A2, a catalytically versatile fungal P450 monooxygenase capable of oxidizing higher-molecular-weight polycyclic aromatic hydrocarbons, alkylphenols, and alkanes. Appl Environ Microbiol 79(8):2692–2702

Teixeira MM, Moreno LF, Stielow BJ, Muszewska A, Hainaut M, Gonzaga L, Abouelleil A, Patané JSL, Priest M, Souza R, Young S, Ferreira KS, Zeng Q, da Cunha MML, Gladki A, Barker B, Vicente VA, de Souza EM, Almeida S, Henrissat B, Vasconcelos ATR, Deng S, Voglmayr H, Moussa TAA, Gorbushina A, Felipe MSS, Cuomo CA, de Hoog GS (2017) Exploring the genomic diversity of black yeasts and relatives (*Chaetothyriales*, *Ascomycota*). Stud Mycol 86:1–28

Tetsch L, Bend J, Hölker U (2006) Molecular and enzymatic characterisation of extra- and intracellular laccases from the acidophilic ascomycete *Hortaea acidophila*. Antonie Van Leeuwenhoek 90(2):183–194

Toledo AV, Virla E, Humber RA, Paradell SL, Lastra CCL (2006) First record of *Clonostachys rosea* (*Ascomycota*: *Hypocreales*) as an entomopathogenic fungus of *Oncometopia tucumana* and *Sonesimia grossa* (*Hemiptera*: *Cicadellidae*) in Argentina. J Invertebr Pathol 92(1):7–10

Tomaselli Scotti C, Durand A (2000) Soil bioremediation by a fungal inoculum of *Cunninghamella elegans* produced by solid state cultivation. Agro Food Ind Hi Tech 11(4):37–40

Trippe KM, Wolpert TJ, Hyman MR, Ciuffetti LM (2014) RNAi silencing of a cytochrome P450 monoxygenase disrupts the ability of a filamentous fungus, *Graphium* sp., to grow on short-chain gaseous alkanes and ethers. Biodegradation 25(1):137–151

van den Brink HJM, van Gorcom RFM, van den Hondel CAMJJ, Punt PJ (1998) Cytochrome P450 enzyme systems in fungi. Fungal Genet Biol 23(1):1–17

van Groenestijn JW, Liu JX (2002) Removal of alpha-pinene from gases using biofilters containing fungi. Atmos Environ 36(35):5501–5508

van Groenestijn JW, van Heiningen WNM, Kraakman NJR (2001) Biofilters based on the action of fungi. Water Sci Technol 44(9):227–232

Vigueras G, Shirai K, Hernández-Guerrero M, Morales M, Revah S (2014) Growth of the fungus *Paecilomyces lilacinus* with *n*-hexadecane in submerged and solid-state cultures and recovery of hydrophobin proteins. Process Biochem 49(10):1606–1611

Voglmayr H, Mayer V, Maschwitz U, Moog J, Djieto-Lordon C, Blatrix R (2011) The diversity of ant-associated black yeasts: insights into a newly discovered world of symbiotic interactions. Fungal Biol 115(10):1077–1091

Wainwright M (1993) In: Jennings DH (ed) Stress tolerance of fungi. Marcel Dekker, New York, pp 127–144

Wang W-J, Wang X-L, Li Y, Xiao S-R, Kepler RM, Yao Y-J (2012) Molecular and morphological studies of *Paecilomyces sinensis* reveal a new clade in clavicipitaceous fungi and its new systematic position. Syst Biodivers 10(2):221–232

Weber FJ, Hage KC, de Bont JAM (1995) Growth of the fungus *Cladosporium sphaerospermum* with toluene as the sole carbon and energy source. Appl Environ Microbiol 61(10):3562–3566

Winquist E, Björklöf K, Schultz E, Räsänen M, Salonen K, Anasonye F, Cajthaml T, Steffen KT, Jørgensen KS, Tuomela M (2014) Bioremediation of PAH-contaminated soil with fungi – from laboratory to field scale. Int Biodeter Biodegr 86(Part C):238–247

Woertz JR, Kinney KA, McIntosh NDP (2001) Removal of toluene in a vapor-phase bioreactor containing a strain of the dimorphic black yeast *Exophiala lecanii-corni*. Biotechnol Bioeng 75:550–558

Wolf HJ, Hanson RS (1980) Identification of methane-utilizing yeasts. FEMS Microbiol Lett 7(2):177–179

Wu Y-R, Luo Z-H, Kwok-Kei Chow R, Vrijmoed LLP (2010) Purification and characterization of an extracellular laccase from the anthracene-degrading fungus *Fusarium solani* MAS2. Bioresour Technol 101(24):9772–9777

Yadav JS, Reddy CA (1993) Degradation of benzene, toluene, ethylbenzene and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 59(3):756–762

Yemashova NA, Murygina VP, Zhukov DV, Zakharyantz AA, Gladchenko MA, Appanna V, Kalyuzhnyi SV (2007) Biodeterioration of crude oil and oil derived products: a review. Rev Environ Sci Biotechnol 6(4):315–337

Zhang D, Yang Y, Leakey JEA, Cerniglia CE (1996) Phase I and phase II enzymes produced by *Cunninghamella elegans* for the metabolism of xenobiotics. FEMS Microbiol Lett 138(2–3):221–226

Zhao J, Zeng J, de Hoog G, Attili-Angelis D, Prenafeta-Boldú F (2010) Isolation and identification of black yeasts by enrichment on atmospheres of monoaromatic hydrocarbons. Microb Ecol 60(1):149–156

ZoBell CE (1946) Action of microorganisms on hydrocarbons. Bacteriol Rev 10:1–49

Index

T. J. McGenity (ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-030-14785-3