

Contents

Preface — v

Contributing authors — xv

R. John Parkes, Henrik Sass, Barry Cragg, Gordon Webster, Erwan Roussel, and Andrew Weightman

1 Studies on prokaryotic populations and processes in subseafloor sediments – an update — 1

- 1.1 New sites investigated — 1
 - 1.1.1 Southeast Atlantic sector of the Southern Ocean (Leg 177) — 1
 - 1.1.2 Woodlark Basin, near Papua New Guinea, Pacific Ocean (Leg 180) — 4
 - 1.1.3 Leg 185, Site 1149 in the Izu-Bonin Trench, Western Equatorial Pacific — 6
 - 1.1.4 Nankai Trough (Leg 190), subduction zone/accretionary prism, Pacific Ocean — 7
 - 1.1.5 Eastern Equatorial Pacific and Peru Margin Sites 1225–1231 (Leg 201) — 10
 - 1.1.6 Newfoundland Margin (Leg 210) — 12
 - 1.1.7 Carbonate mound (IODP Expedition 307) — 13
- 1.2 High-pressure cultivation – DeepIsoBUG, gas hydrate sediments — 15
- 1.3 Subseafloor biosphere simulation experiments — 18
- 1.4 Conclusions — 20

Jennifer F. Biddle, Sean P. Jungbluth, Mark A. Lever, and Michael S. Rappé

2 Life in the Oceanic Crust — 29

- 2.1 Introduction — 29
- 2.2 Sampling tools — 30
 - 2.2.1 Tools for accessing the deep basement biosphere — 32
- 2.3 Contamination — 36
 - 2.3.1 Contamination induced during drilling — 36
 - 2.3.2 Contamination during fluid sampling — 38
- 2.4 Direct evidence for life in the deep ocean crust — 38
 - 2.4.1 Textural alterations — 39
 - 2.4.2 Geochemical evidence from fluids — 40
 - 2.4.3 Geochemical evidence from rocks — 41
 - 2.4.4 Genetic surveys — 45
- 2.5 Future directions — 51

Karsten Pedersen

3 Microbial life in terrestrial hard rock environments — 63

- 3.1 Hard rock aquifers from the perspective of microorganisms — 63
- 3.2 Windows into the terrestrial hard rock biosphere — 64
 - 3.2.1 Sampling methods for microbes in hard rock aquifers — 64
 - 3.2.2 Yesterday marine – terrestrial today — 65
 - 3.2.3 Basalts and ophiolites — 66
 - 3.2.4 Granites — 68
 - 3.2.5 Hard rocks of varying origin — 70
- 3.3 Energy from where? — 71
 - 3.3.1 Deep reduced gases — 72
- 3.4 Activity — 73
 - 3.4.1 Stable isotopes — 73
 - 3.4.2 Geochemical indicators — 74
 - 3.4.3 *In vitro* activity — 74
 - 3.4.4 *In situ* activity — 74
 - 3.4.5 Phages may control activity rates — 76
- 3.5 What's next in the exploration of microbial life in deep hard rock aquifers? — 76

Laurent Toffin, Karine Alain

4 Technological state of the art and challenges — 83

- 4.1 Basic concepts and difficulties inherent to the cultivation of subseafloor prokaryotes — 83
- 4.2 Microbial growth monitoring, method detection limits and innovative cultivation methods — 91
- 4.3 Challenges and research needs (instrumental, methodological and logistics needs) — 92

Yuki Morono, Motoo Ito, and Fumio Inagaki

5 Detecting slow metabolism in the subseafloor: analysis of single cells using NanoSIMS — 101

- 5.1 Introduction — 101
- 5.2 Overview of ion imaging with a NanoSIMS ion microprobe — 102
- 5.3 Detecting slow metabolism: bulk to single cells — 105
 - 5.3.1 Bulk measurement of subseafloor microbial activity using radiotracers — 105
 - 5.3.2 Observing radioactive substrate incorporation at the cellular level: microautoradiography — 106
 - 5.3.3 Quantitative analysis of stable isotope incorporation using NanoSIMS — 107

- 5.4 Bridging identification and functional analysis of microbes using elemental labeling — **110**
- 5.5 Critical step for successful NanoSIMS analysis: sample preparation — **112**
- 5.6 Future directions — **114**

Karen G. Lloyd

- 6 Quantifying microbes in the marine subseafloor: some notes of caution — 121**
- 6.1 Introduction — **121**
- 6.2 Quantification of specific microbial groups in marine sediments — **124**
- 6.3 Assessment of quantitative methods in marine sediments: the Leg 201 Peru Margin example — **128**
- 6.4 Global meta-analysis of FISH, CARD-FISH and qPCR quantifications of bacteria and archaea — **132**
- 6.5 Future outlook — **134**

Andreas Teske

- 7 *Archaea* in deep marine subsurface sediments — 143**
- 7.1 Introduction — **143**
- 7.2 Archaeal Ribosomal RNA phylogeny — **143**
- 7.3 Marine subsurface *Archaea* — **144**
- 7.4 Archaeal habitat preferences in the subsurface — **149**
- 7.5 Methanogenic and methane-oxidizing archaea — **152**
- 7.6 Archaeal abundance and ecosystem significance in the subsurface — **154**

Bernard Ollivier, Jean Borgomano, and Philippe Oger

- 8 Petroleum: from formation to microbiology — 161**
- 8.1 Introduction — **161**
- 8.2 Petroleum formation — **161**
- 8.2.1 Petroleum system — **163**
- 8.3 Petroleum microbiology — **166**
- 8.3.1 The sulfate-reducing prokaryotes — **168**
- 8.3.2 The methanoarchaea — **171**
- 8.3.3 The fermentative prokaryotes — **174**
- 8.3.4 Other metabolic lifestyle bacteria — **177**
- 8.4 Conclusion — **179**

Virginia Edgcomb, William Orsi, and Jennifer F. Biddle

9 Fungi in the marine subsurface — 187

- 9.1 Introduction — **187**
- 9.2 The concept of marine fungi — **187**
- 9.3 Fungi in marine near-surface sediments in the deep sea — **189**
- 9.4 Fungi in the deep subsurface — **190**
 - 9.4.1 Initial whole community and prokaryote-focused studies of the marine subsurface yielding information on eukaryotes — **190**
 - 9.4.2 Eukaryote-focused studies yielding information on fungi in the deep subsurface — **191**
- 9.5 How deep do fungi go in the subsurface? — **197**
- 9.6 Summary — **197**

Mashal Alawi

10 Microbes in geo-engineered systems: geomicrobiological aspects of CCS and Geothermal Energy Generation — 203

- 10.1 Introduction — **203**
 - 10.1.1 Carbon Capture and Storage (CCS) — **204**
 - 10.1.2 Geothermal energy and aquifer energy storage — **205**
- 10.2 Microbial diversity in geo-engineered reservoirs — **206**
- 10.3 Interactions between microbes and geo-engineered systems — **208**
 - 10.3.1 General considerations — **208**
 - 10.3.2 Microbial processes in the deep biosphere potentially affected by CCS — **209**
 - 10.3.3 Examples from a CCS pilot site, CO₂ degassing sites and laboratory experiments — **211**
 - 10.3.4 Impact of microbially-driven processes on CO₂ trapping mechanisms — **213**
 - 10.3.5 Impact of microbially-driven processes on CCS facilities — **214**
 - 10.3.6 Impact of microbially-driven processes on geothermal energy plants — **214**
- 10.4 Methods to analyze the interaction between geo-engineered systems and the deep biosphere — **216**
 - 10.4.1 Sampling of reservoir fluids and rock cores — **216**
 - 10.4.2 Methods to analyze microbes in geo-engineered systems — **216**

Charles S. Cockell

11 The subsurface habitability of terrestrial rocky planets: Mars — 225

- 11.1 Introduction — **225**
- 11.2 The subsurface of Mars – our current knowledge — **226**
- 11.3 Martian subsurface habitability, past and present — **233**
 - 11.3.1 Vital elements (C, H, N, O, P, S) — **233**

11.3.2	Other micronutrients and trace elements —	234
11.3.3	Liquid water through time —	235
11.3.4	Redox couples —	238
11.3.5	Radiation —	239
11.3.6	Other physical and environmental factors —	239
11.3.7	Acidity —	240
11.4	Impact craters and deep subsurface habitability —	242
11.5	The near-subsurface habitability of present and recent Mars – an empirical example —	243
11.6	Uninhabited, but habitable subsurface environments? —	245
11.7	Ten testable hypotheses on habitability of the Martian subsurface —	247
11.8	Sampling the subsurface of Mars —	250
11.9	Conclusion —	251

Rolando di Primio

12 Assessing biosphere-geosphere interactions over geologic time scales: insights from Basin Modeling — 261

12.1	Introduction —	261
12.2	Basin Modeling —	262
12.3	Modeling processes at the deep bio-geo interface —	264
12.3.1	Feeding the deep biosphere (biogenic gas) —	264
12.3.2	Petroleum biodegradation —	267
12.4	Modeling processes at the shallow bio-geo interface —	274
12.5	Conclusions —	275

Doug LaRowe, Jan Amend

13 Energetic constraints on life in marine deep sediments — 279

13.1	Introduction —	279
13.2	Previous work —	280
13.3	Study site overview —	280
13.3.1	Juan de Fuca (JdF) —	281
13.3.2	Peru Margin (PM) —	281
13.3.3	South Pacific Gyre (SPG) —	282
13.4	Overview of catabolic potential —	282
13.5	Comparing deep biospheres —	288
13.6	Electron acceptor utilization —	290
13.7	Energy demand —	292
13.8	Concluding remarks —	293
13.9	Computational methods —	293
13.9.1	Thermodynamic properties of anhydrous ferrihydrite and pyrolusite —	294

Hans Røy

14 Experimental assessment of community metabolism in the subsurface — 303

14.1 Introduction — **303**

14.1.1 The energy source — **303**

14.1.2 The carbon budget — **304**

14.1.3 Distribution vertical of microbial metabolism the sediment pile — **305**

14.2 Quantifiable metabolic processes — **306**

14.2.1 Reaction diffusion modeling and mass balances — **307**

14.2.2 Measurements of rates of energy metabolism with exotic isotopes — **312**

14.3 Summary — **315**

Index — 319