

2805-9004

---

# GROUND-WATER MICROBIOLOGY AND GEOCHEMISTRY

---

Second Edition

**Francis H. Chapelle**  
United States Geological Survey



**JOHN WILEY & SONS, INC.**

New York / Chichester / Weinheim / Brisbane / Singapore / Toronto

# CONTENTS

---

Preface	xv
Acknowledgments	xvii
<b>I. OVERVIEW OF MICROBIOLOGY</b>	<b>1</b>
<b>1. HISTORY, GEOLOGY, AND MICROBIOLOGY</b>	<b>3</b>
1.1 Geology—An Observational Science / 4	
1.2 Microbiology—An Experimental Science / 15	
1.3 Ground-Water Chemistry and Subsurface Microbiology / 22	
1.3.1 Subsurface Microbiology and the Geosciences / 23	
1.3.2 Subsurface Microbiology and Microbial Ecology / 26	
1.3.3 Subsurface Microbiology and Contaminant Biodegradation / 28	
1.4 Chapter Summary / 30	
Questions to Consider / 31	
<b>2. MICROORGANISMS PRESENT IN THE GROUND-WATER ENVIRONMENT</b>	<b>32</b>
2.1 The Bacteria / 33	

- 2.2 The Eucarya / 36
  - 2.2.1 Eucaryotes in Ground-Water Systems / 38
- 2.3 The Archaea / 39
- 2.4 The Viruses / 41
  - 2.4.1 Viral Ecology / 43
  - 2.4.2 Viruses in Ground-Water Systems / 44
- 2.5 Bacteria in Ground-Water Systems / 45
  - 2.5.1 Classifying Bacteria / 46
  - 2.5.2 Gram-Negative Bacteria Found in Ground-Water Systems / 50
  - 2.5.3 Gram-Positive Bacteria in Ground-Water Systems / 55
- 2.6 Chapter Summary / 56
- Questions to Consider / 57

### **3. BACTERIAL GROWTH**

**58**

- 3.1 Bacterial Reproduction / 58
- 3.2 Population Growth Kinetics / 60
- 3.3 Environmental Conditions and Bacterial Growth / 62
  - 3.3.1 Temperature / 63
  - 3.3.2 Water / 65
  - 3.3.3 Molecular Oxygen / 66
  - 3.3.4 pH / 66
  - 3.3.5 Osmotic Pressure / 67
- 3.4 Techniques for Culturing Bacteria / 68
  - 3.4.1 Design of Growth Media / 69
  - 3.4.2 Isolating Bacteria from Environmental Samples / 73
- 3.5 Enumerating Bacteria / 77
  - 3.5.1 Viable Counting Procedures / 77
  - 3.5.2 Direct Counting Procedures / 79
- 3.6 Chapter Summary / 80
- Questions to Consider / 81

### **4. BACTERIAL METABOLISM**

**82**

- 4.1 Thermodynamics and Bacterial Metabolism / 82
- 4.2 ATP Synthesis—Storing Energy / 84
- 4.3 Electron Transport Systems—Releasing Energy / 86
- 4.4 Chemiosmosis—Harnessing Energy from Electron Transport / 88
- 4.5 The Role of Enzymes / 88

- 4.6 Energy-Releasing Pathways of Geochemical Importance / 92
  - 4.6.1 Lactate and Acetate Fermentations / 93
  - 4.6.2 Ferredoxins and the Production of Hydrogen and Acetate in Fermentation / 94
  - 4.6.3 Methanogenic Pathways / 96
  - 4.6.4 Sulfate Reduction / 98
  - 4.6.5 Fe(III) Reduction / 99
  - 4.6.6 Nitrate Reduction / 100
  - 4.6.7 Oxygen Reduction—Aerobic Metabolism / 102
- 4.7 Biosynthesis / 103
  - 4.7.1 Amino Acids / 104
  - 4.7.2 Carbohydrates / 104
  - 4.7.3 Lipids / 106
- 4.8 Chemolithotrophy / 107
  - 4.8.1 Hydrogen Oxidizers / 107
  - 4.8.2 Sulfide Oxidizers / 108
  - 4.8.3 Iron Oxidizers / 110
  - 4.8.4 Ammonia-Oxidizing (Nitrifying) Bacteria / 110
  - 4.8.5 Autotrophic CO<sub>2</sub> Fixation / 111
- 4.9 Metabolic Control of Geochemical Processes / 112
- 4.10 Summary / 114
- Questions to Consider / 115

## **5. BACTERIAL GENETICS**

**116**

- 5.1 DNA—Its Structure and Organization / 117
- 5.2 RNA—Its Structure and Organization / 120
  - 5.2.1 Transcription / 120
  - 5.2.2 Translation—Making Proteins / 120
- 5.3 Gene Expression and Regulation / 123
  - 5.3.1 Induction / 123
  - 5.3.2 Repression / 123
- 5.4 Mutations / 124
  - 5.4.1 Mutagenic Agents / 125
  - 5.4.2 Transposable Genetic Material / 126
- 5.5 Natural Genetic Exchanges / 127
  - 5.5.1 Recombination / 127
- 5.6 DNA Technology / 128
  - 5.6.1 Analyzing DNA / 129
  - 5.6.2 DNA Cloning / 132
- 5.7 Genetic Engineering / 137

- 5.7.1 Plasmids / 137
- 5.7.2 Vectors / 138
- 5.8 Applications of DNA Technology / 140
  - 5.8.1 Insulin Production / 140
  - 5.8.2 Enhanced Biodegradation / 140
- 5.9 DNA Technology in Subsurface Microbiology / 142
  - 5.9.1 Phylogenetic Analysis of Fe(III)-Reducing Bacteria / 142
  - 5.9.2 Using DNA Probes to Find Contaminant-Degrading Microorganisms / 145
  - 5.9.3 Release of Genetically Engineered Microorganisms to the Environment / 146
- 5.10 Summary / 147
- Questions to Consider / 148

**6. MICROBIAL ECOLOGY OF GROUND-WATER SYSTEMS 150**

- 6.1 Scope of Subsurface Microbial Ecology / 152
- 6.2 Methods in Subsurface Microbial Ecology / 155
  - 6.2.1 Culture Methods / 156
  - 6.2.2 Direct Observation / 159
  - 6.2.3 Phospholipid Fatty Acid Analysis / 161
  - 6.2.4 Activity Measurements in Microcosms / 163
  - 6.2.5 Geochemical Methods / 166
  - 6.2.6 Molecular Microbial Ecology / 168
- 6.3 Microbial Diversity and Niches in Aquifer Systems / 171
  - 6.3.1 Measurement of Diversity / 171
  - 6.3.2 Niches and Sources of Microbial Diversity / 172
  - 6.3.3 Stress and Microbial Diversity / 174
- 6.4 Population Interactions / 174
  - 6.4.1 Neutralism / 175
  - 6.4.2 Commensalism / 175
  - 6.4.3 Synergism and Symbiosis / 176
  - 6.4.4 Competition / 177
  - 6.4.5 Antagonism, Parasitism, and Predation / 180
- 6.5 r and K Strategies in Microbial Ecosystems / 181
  - 6.5.1 r and K Strategies in the Aquifer Environment / 182
- 6.6 Chapter Summary / 183
- Questions to Consider / 184

II. MICROBIAL PROCESSES IN PRISTINE GROUND-WATER SYSTEMS	187
<b>7. ABUNDANCE AND DISTRIBUTION OF BACTERIA IN THE SUBSURFACE</b>	<b>189</b>
7.1 Classification of Subsurface Environments / 189	
7.2 The Unsaturated Zone / 193	
7.2.1 The Unsaturated Zone As a Microbial Habitat / 195	
7.2.2 Biomass Measurements in Soil Microbiology / 197	
7.2.3 Distribution of Bacteria in the Unsaturated Zone / 201	
7.3 Local Flow Systems / 202	
7.3.1 Local Flow Systems as a Microbial Habitat / 203	
7.3.2 Distribution of Bacteria in Local Flow Systems / 205	
7.4 Intermediate Flow Systems / 209	
7.4.1 Intermediate Flow Systems As a Microbial Habitat / 209	
7.4.2 Distribution of Bacteria in Intermediate Flow Systems / 210	
7.4.3 Microbial Processes in Confining Beds / 214	
7.5 Regional Flow systems / 217	
7.5.1 Early Observations from Petroleum Reservoirs / 218	
7.5.2 Distribution of Bacteria in Regional Flow Systems / 219	
7.7 Chapter Summary / 221	
Questions to Consider / 222	
<b>8. MICROBIOLOGICAL SAMPLING OF SUBSURFACE ENVIRONMENTS</b>	<b>223</b>
8.1 Sampling the Unsaturated Zone / 224	
8.1.1 Hand Augering / 224	
8.1.2 Air Drilling and Coring / 225	
8.2 Sampling Local Flow Systems / 226	
8.2.1 Split Spoon Sampling / 226	
8.2.2 Push-Tube (Shelby Tube) Sampling Methods / 227	
8.2.3 Direct Push Sampling / 229	
8.2.4 Aseptic Technique with Split-Spoon, Shelby Tube, and Direct Push Sampling / 229	
8.3 Sampling Intermediate and Regional Systems / 231	

8.3.1	Mud Rotary Drilling / 231	
8.3.2	Drilling Fluids / 232	
8.3.3	Mud-Rotary Coring / 236	
8.4	Drilling Fluid Contamination of Cored Sediments / 240	
8.4.1	Down-Hole Saturation Contamination / 240	
8.4.2	Core Seepage Contamination / 241	
8.4.3	Core-fracture Contamination / 241	
8.4.4	Evaluating Drilling Fluid Contamination / 242	
8.5	Sampling Ground Water for Microorganisms / 246	
8.6	Chapter Summary / 247	
	Questions to Consider / 248	
<b>9.</b>	<b>BIOGEOCHEMICAL CYCLING IN GROUND-WATER SYSTEMS</b>	<b>250</b>
9.1	The Oxygen Cycle / 251	
9.1.1	Oxygen Cycling in Ground-Water Systems / 252	
9.2	The Carbon Cycle / 255	
9.2.1	The Integrated Carbon, Oxygen, and Hydrogen Cycles / 257	
9.2.2	Carbon Cycling in Ground-Water Systems / 258	
9.3	The Nitrogen Cycle / 261	
9.3.1	Nitrogen Cycling in Ground-Water Systems / 264	
9.4	The Iron Cycle / 270	
9.4.1	Iron Cycling in Aquatic Sediments / 271	
9.4.2	Iron Cycling in Ground-Water Systems / 272	
9.5	The Sulfur Cycle / 275	
9.5.1	Sulfur Cycling in Ground-Water Systems / 276	
9.6	Chapter Summary / 280	
	Questions to Consider / 281	
<b>10.</b>	<b>OXIDATION-REDUCTION PROCESSES IN GROUND-WATER SYSTEMS</b>	<b>282</b>
10.1	Overview of Redox Geochemistry / 283	
10.1.1	The Equilibrium Approach / 283	
10.1.2	The Kinetic Approach / 286	
10.1.3	Redox Processes in Ground-Water Systems / 286	
10.2	Describing Kinetic Redox Processes in Ground-Water Systems / 288	
10.2.1	Identifying Electron Donors / 288	
10.2.2	Identifying Electron Acceptors / 289	
10.3	Identifying Terminal Electron-Accepting Processes (TEAPs) in the Environment / 295	

- 10.3.1 Redox Zonation in Aquatic Sediments / 295
- 10.3.2 Redox Zonation in Ground-Water Systems / 296
- 10.4 Redox Processes in Pristine Ground-Water Systems / 298
  - 10.4.1 Black Creek Aquifer of South Carolina / 298
  - 10.4.2 Floridan Aquifer of Georgia / 301
- 10.5 Redox Processes in Contaminated Ground-Water Systems / 305
  - 10.5.1 A Petroleum Hydrocarbon–Contaminated Aquifer, Charleston, South Carolina / 305
  - 10.5.2 A Mixed Petroleum Hydrocarbon/Chlorinated Solvent–Contaminated Aquifer, Plattsburgh, New York / 307
  - 10.5.3 Complications Associated with Small-Scale Redox Zones / 309
- 10.6 Kinetic Modeling of Redox Processes / 314
  - 10.6.1 Electron Flow in Ground-Water Systems / 314
  - 10.6.2 Developing Kinetic Models of Microbial Redox Processes / 315
- 10.7 Chapter Summary / 319
- Questions to Consider / 319

III.	<b>MICROBIAL PROCESSES IN CONTAMINATED GROUND-WATER SYSTEMS</b>	<b>321</b>
11.	<b>MICROBIAL ACCLIMATION TO GROUND-WATER CONTAMINATION</b>	<b>323</b>
	11.1 Microbial Response to Environmental Changes / 324	
	11.2 Mechanisms of Acclimation / 325	
	11.2.1 Induction / 326	
	11.2.2 Catabolite Repression / 327	
	11.2.3 Genetic Mutations / 328	
	11.2.4 Acclimation to Available Electron Acceptors / 329	
	11.2.5 Production of Proteins in Response to Chemical Stresses / 331	
	11.3 Factors Affecting Microbial Acclimation / 332	
	11.3.1 Rates of Acclimation / 332	
	11.3.2 Concentration Effects / 333	
	11.3.3 Cross-Acclimation of Xenobiotic Compounds / 336	
	11.3.4 Chemical Structure of Xenobiotics / 336	
	11.4 Acclimation to Xenobiotics in Ground-Water Systems / 337	
	11.4.1 Acclimation Response in a Contaminated Aquifer / 338	



- 11.4.2 Acclimation Response in Pristine Aquifer Sediments / 338
- 11.4.3 Acclimation of Eucaryotic Microorganisms / 340
- 11.4.4 Acclimation in Bioremediation Technology / 342
- 11.5 Acclimation to Metal Toxicity / 342
  - 11.5.1 Metal Detoxification Mechanisms / 343
  - 11.5.2 Plasmid-Encoded Metal Resistance Mechanisms / 344
  - 11.5.3 Acclimation to Mercury Toxicity / 345
- 11.6 Summary / 346
- Questions to Consider / 347

**12. BIODEGRADATION AND BIOREMEDIATION OF PETROLEUM HYDROCARBONS IN GROUND-WATER SYSTEMS**

**349**

- 12.1 Composition of Crude Oil / 349
- 12.2 Petroleum Refining and Fuel Blending / 350
- 12.3 Movement and Separation of Petroleum Hydrocarbons in Ground-Water Systems / 353
  - 12.3.1 Density-Driven Migration of Hydrocarbons / 353
  - 12.3.2 Solubility and Hydrocarbon Separation in Ground-Water Systems / 355
- 12.4 Microbial Degradation of Aliphatic Hydrocarbons / 356
  - 12.4.1 Methane Oxidation / 357
  - 12.4.2 Oxidation of n-alkanes / 358
  - 12.4.3 Alkene Oxidation and Reduction / 360
  - 12.4.4 Branched Aliphatics / 361
- 12.5 Microbial Degradation of Alicyclic Hydrocarbons / 361
  - 12.5.1 Pathways for Cyclohexanol Degradation / 362
- 12.6 Microbial Degradation of Aromatic Hydrocarbons / 363
  - 12.6.1 Benzene Degradation / 364
  - 12.6.2 Degradation of Alkyl Benzenes / 365
  - 12.6.3 Degradation of Polycyclic Aromatic Compounds / 367
  - 12.6.4 Anaerobic Degradation of Aromatic Hydrocarbons / 368
  - 12.6.5 Biodegradation of Alkyl Ethers / 372
- 12.7 Microbial Degradation of Petroleum Hydrocarbons in Ground-Water Systems / 375
  - 12.7.1 Aerobic Degradation of BTX Compounds / 376
  - 12.7.2 Anaerobic Degradation of BTEX Compounds / 378

- 12.8 Bioremediation of Petroleum Hydrocarbon Contamination in Ground-Water Systems / 379
  - 12.8.1 Marine Oil Spills and the Biodegradation of Petroleum Hydrocarbons / 380
  - 12.8.2 Microbial Degradation Processes in Shallow Water Table Aquifers / 382
  - 12.8.3 Engineered Bioremediation of Petroleum Hydrocarbons in Ground-Water Systems / 383
  - 12.8.4 Monitored Natural Attenuation of Petroleum Hydrocarbons / 390
  - 12.8.5 Estimating Biodegradation Rates in Ground-Water Systems / 392
- 12.9 Summary / 401
- Questions to Consider / 402

### **13. BIODEGRADATION AND BIOREMEDIATION OF HALOGENATED ORGANIC COMPOUNDS IN GROUND-WATER SYSTEMS**

**403**

- 13.1 Chemistry and Uses of Halogenated Organic Compounds / 403
  - 13.1.1 Aliphatic Compounds / 404
  - 13.1.2 Monocyclic Aromatic Compounds / 407
  - 13.1.3 Polychlorinated Biphenols / 409
  - 13.1.4 Organochlorine Insecticides / 409
  - 13.1.5 Chlorinated Herbicides / 410
  - 13.1.6 Chlorinated Phenols / 411
- 13.2 Microbial Degradation of Halogenated Organic Compounds / 412
  - 13.2.1 Chlorinated Ethenes / 413
  - 13.2.2 Chlorinated Benzenes / 420
  - 13.2.3 Polychlorinated Biphenyls / 421
  - 13.2.4 Organochlorine Insecticides / 422
  - 13.2.5 Chlorinated Herbicides / 423
  - 13.2.6 Chlorinated Phenols / 424
- 13.3 Biodegradation of Halogenated Organic Compounds in Ground-Water Systems / 425
  - 13.3.1 Reductive and Oxidative Biodegradation Patterns for Chlorinated Ethenes / 426
  - 13.3.2 Cometabolic Degradation of Chlorinated Ethenes / 433
  - 13.3.3 Degradation Patterns of Alkyl Halide Insecticides / 437
  - 13.3.4 Degradation Patterns of Chlorobenzenes / 438

13.3.5	Degradation of Chlorinated Herbicides /	439
13.3.6	Degradation of Chlorophenolic Compounds /	441
13.4	Summary /	443
	Questions to Consider /	444
	References	446
	Index	469