

# **Advances in MICROBIAL ECOLOGY**

---

**Volume 8**

**Edited by**

**K. C. Marshall**

# **Advances in MICROBIAL ECOLOGY**

---

**Volume 8**

**Edited by**

**K. C. Marshall**

*University of New South Wales  
Kensington, New South Wales, Australia*

**PLENUM PRESS • NEW YORK AND LONDON**

The Library of Congress cataloged the first volume of this title as follows:

---

Advances in microbial ecology. v. 1-  
New York, Plenum Press c1977-  
v. ill. 24 cm.

Key title: Advances in microbial ecology, ISSN 0147-4863

1. Microbial ecology--Collected works.

QR100.A36

576'.15

77-649698

---

Library of Congress Catalog Card Number 77-649698  
ISBN 0-306-41877-0

© 1985 Plenum Press, New York  
A Division of Plenum Publishing Corporation  
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

# Advances in MICROBIAL ECOLOGY

Volume 8

R. M. ADAMS

University of Kentucky  
Lexington, Kentucky

B. B. JORGENSEN

University of Aarhus  
Aarhus, Denmark

J. H. JENSEN

University of North Carolina at Chapel Hill  
Chapel Hill, North Carolina

For a complete list of titles in this series, see the back cover of the book. A complete list of titles in this series is also available on request. For a complete list of titles in this series, see the back cover of the book. A complete list of titles in this series is also available on request.

## ADVANCES IN MICROBIAL ECOLOGY

---

Sponsored by the International Committee on Microbial Ecology (ICOME), a committee of the International Union of Microbiological Societies (IUMS) and the International Union of Biological Sciences (IUBS)

### EDITORIAL BOARD

**R. M. Atlas**

*University of Louisville  
Louisville, Kentucky*

**B. B. Jørgensen**

*University of Aarhus  
Aarhus, Denmark*

**J. H. Slater**

*University of Wales Institute of Science & Technology  
Cardiff, Wales, United Kingdom*

---

A Continuation Order Plan is available for this series. A continuation order will bring delivery of each new volume immediately upon publication. Volumes are billed only upon actual shipment. For further information please contact the publisher.

## Contributors

**Martin Alexander**, Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14853

**Trevor Duxbury**, Department of Microbiology, University of Sydney, Sydney, New South Wales 2006, Australia

**Karl-Erik Eriksson**, Swedish Forest Products Research Laboratory, S-11486, Stockholm, Sweden

**Hans van Gernerden**, Laboratorium voor Microbiologie, University of Groningen, Haren, The Netherlands

**J. Gijs Kuenen**, Laboratorium voor Microbiologie, Delft University of Technology, Delft, The Netherlands

**Adrian Lee**, School of Microbiology, University of New South Wales, Kensington, New South Wales 2033, Australia

**Lars G. Ljungdahl**, Center for Biological Resource Recovery, Department of Biochemistry, University of Georgia, Athens, Georgia 30602

**Lesley A. Robertson**, Laboratorium voor Microbiologie, Delft University of Technology, Delft, The Netherlands

**Joseph A. Robinson**, The UpJohn Company, Kalamazoo, Michigan 49001

## Preface

*Advances in Microbial Ecology* was established by the International Committee on Microbial Ecology (ICOME) as a vehicle for the publication of critical reviews selected to reflect current trends in the ever-expanding field of microbial ecology. Most of the chapters found in *Advances in Microbial Ecology* have been solicited by the Editorial Board. Individuals are encouraged, however, to submit outlines of unsolicited contributions to any member of the Editorial Board for consideration for inclusion in a subsequent volume of *Advances*. Contributions are expected to be in-depth, even provocative, reviews of topical interest relating to the ecology of microorganisms.

With the publication of Volume 8 of *Advances* we welcome to the panel of contributors Martin Alexander, the founding editor of this series, who discusses the range of natural constraints on nitrogen fixation in agricultural ecosystems. Ecological aspects of cellulose degradation are discussed by L. G. Ljungdahl and K.-E. Eriksson, and of heavy metal responses in microorganisms by T. Duxbury. In his chapter, A. Lee considers the gastrointestinal tract as an ecological system, and comments on the possibility of manipulating this system. The complex interactions among aerobic and anaerobic sulfur-oxidizing bacteria are discussed in terms of natural habitats and chemostat culture by J. G. Kuenen, L. Robertson, and H. van Gernerden. Finally, J. A. Robinson presents the advantages and limitations in the use of nonlinear regression analysis in determining microbial kinetic parameters in ecological situations.

K. C. Marshall, Editor  
R. M. Atlas  
B. B. Jørgensen  
J. H. Slater

# Contents

## Chapter 1

### Microbial Interactions among Aerobic and Anaerobic Sulfur-Oxidizing Bacteria

J. Gijs Kuenen, Lesley A. Robertson, and Hans van Gernerden

|   |    |
|---|----|
| 1. Introduction   | 1  |
| 2. The Types of Bacteria  | 5  |
| 2.1. The Colorless Sulfur Bacteria                                  | 5  |
| 2.2. The Phototrophic Sulfur Bacteria                               | 11 |
| 3. Types of Microbial Interaction                                   | 15 |
| 3.1. Neutralism   | 16 |
| 3.2. Mutualism  | 16 |
| 3.3. Commensalism   | 16 |
| 3.4. Amensalism   | 17 |
| 3.5. Predation  | 17 |
| 3.6. Competition  | 17 |
| 4. Competition  | 17 |
| 4.1. Competition Involving Chemolithotrophs                         | 19 |
| 4.2. Competition Involving Phototrophs                              | 28 |
| 4.3. Other Selective Pressures                                      | 38 |
| 4.4. Competition between Colorless and Phototrophic Sulfur Bacteria | 40 |
| 5. Other Interactions Involving the Sulfide-Oxidizing Bacteria      | 44 |
| 5.1. Examples among the Chemolithotrophs                            | 44 |
| 5.2. Examples among the Phototrophs                                 | 47 |
| 6. Enrichment Studies   | 50 |
| 6.1. Aerobic Enrichments of Colorless Sulfur Bacteria               | 50 |
| 6.2. Anaerobic Enrichments of Sulfide-Oxidizing Bacteria            | 50 |
| 7. Conclusion   | 52 |
| References  | 54 |



## Chapter 2

### **Determining Microbial Kinetic Parameters Using Nonlinear Regression Analysis: Advantages and Limitations in Microbial Ecology**

Joseph A. Robinson

|  |     |
|--|-----|
| 1. Introduction .....  | 61  |
| 2. Fundamental Definitions .....   | 62  |
| 2.1. Parameters versus Variables .....   | 63  |
| 2.2. Linear versus Nonlinear Models .....  | 63  |
| 3. Use of Linearized Forms of Nonlinear Models .....   | 64  |
| 3.1. Rationale .....   | 64  |
| 3.2. Limitations .....   | 64  |
| 4. Parameter Estimation Strategies .....   | 69  |
| 4.1. The Function to Be Minimized .....  | 69  |
| 4.2. Least Squares Estimation .....  | 70  |
| 4.3. Methods of Minimizing Equation (6) for Nonlinear Models .....                                       | 71  |
| 4.4. Weighted Least Squares (WLS) Analysis .....   | 81  |
| 4.5. Robust Regression .....   | 85  |
| 4.6. Alternative Objective Functions for the Estimation of Microbial Parameters .....                    | 87  |
| 4.7. Jackknife Estimation .....  | 88  |
| 4.8. Bootstrapping .....   | 90  |
| 5. Residuals Analysis .....  | 91  |
| 5.1. Influence of Correlated Measurement Errors on Parameters Estimated via Least Squares Analysis ..... | 92  |
| 5.2. Number of Data Points Required for Detection of Correlated Errors .....                             | 92  |
| 6. Model Discrimination .....  | 92  |
| 7. Optimal Experimental Design .....   | 95  |
| 7.1. Optimal Experiments for Model Discrimination .....  | 95  |
| 7.2. Optimal Experiments for Parameter Estimation .....  | 96  |
| 7.3. Experiments of Optimal Duration .....   | 98  |
| 8. Some Models of Interest to Microbial Ecologists .....   | 99  |
| 8.1. Exponential Models .....  | 99  |
| 8.2. Michaelis-Menten Model .....  | 101 |
| 8.3. Other Uptake Models .....   | 104 |
| 8.4. Growth Models .....   | 105 |
| 8.5. Models with More Than One Independent Variable .....  | 108 |
| 9. Concluding Remarks .....  | 109 |
| References .....   | 110 |

## Chapter 3

**Neglected Niches: The Microbial Ecology of the Gastrointestinal Tract**

Adrian Lee

|   |     |
|---|-----|
| 1. Introduction .....   | 115 |
| 2. Species Diversity in the Gastrointestinal Tract .....  | 116 |
| 2.1. Factors Influencing Species Diversity .....  | 117 |
| 2.2. The Host as a Contributor to and a Beneficiary of the<br>Microbial Ecosystem of the Intestinal Tract ..... | 122 |
| 3. Colonization Resistance .....  | 123 |
| 3.1. Competition for Nutrients .....  | 125 |
| 3.2. Toxic Metabolites .....  | 126 |
| 3.3. Bacterial Barriers .....   | 127 |
| 4. Perturbation of the Gut Ecosystem .....  | 127 |
| 4.1. Consequences of the Vacated Niche .....  | 128 |
| 4.2. Examples of Natural Perturbation .....   | 128 |
| 4.3. Experimental Perturbations of the Gut Ecosystem .....  | 132 |
| 5. Consequences of an Immature Microbiota .....   | 134 |
| 5.1. The Neonatal Animal .....  | 134 |
| 5.2. The Infant Bowel .....   | 136 |
| 5.3. Specific Pathogen Free (SPF) Animals .....   | 138 |
| 6. Immune Mechanisms .....  | 140 |
| 7. Adaptation to the Ecosystem: Important Determinants of<br>Microbial Pathogenicity .....                      | 142 |
| 7.1. Adhesion to Intestinal Surfaces .....  | 143 |
| 7.2. Mucus Colonization .....   | 143 |
| 7.3. Nutrient Avidity .....   | 146 |
| 8. Behaviorism in the Intestinal Tract: The Importance of What<br>Organisms Do Rather Than What They Are .....  | 147 |
| 9. Man versus Mouse: The Relevance of Current Concepts of<br>Intestinal Ecology .....                           | 150 |
| 10. Manipulation of the Intestinal Ecosystem .....  | 152 |
| 10.1. Replacement or Preseeding of the Premature or<br>Perturbed Gut Ecosystem .....                            | 152 |
| 10.2. Selective Decontamination of the Digestive Tract .....  | 154 |
| 10.3. Environmental Manipulation of the Gut Ecosystem .....   | 155 |
| 11. Conclusion .....  | 155 |
| References .....  | 156 |

## Chapter 4

**Ecological Constraints on Nitrogen Fixation in Agricultural Ecosystems**

Martin Alexander

|  |     |
|--|-----|
| 1. Introduction                                      | 163 |
| 2. The <i>Rhizobium</i> Inoculum                     | 164 |
| 3. Establishment of <i>Rhizobium</i>                 | 166 |
| 4. Survival of <i>Rhizobium</i> in Soil              | 168 |
| 5. Biological Stresses on <i>Rhizobium</i>           | 169 |
| 6. Abiotic Stresses on <i>Rhizobium</i>              | 171 |
| 6.1. Acidity   | 171 |
| 6.2. Desiccation                                     | 173 |
| 6.3. Temperature                                     | 173 |
| 6.4. Salinity and Alkalinity                         | 175 |
| 6.5. Fungicides                                      | 175 |
| 7. Limitations on Nitrogen Fixation by Cyanobacteria | 176 |
| 8. Conclusion  | 178 |
| References   | 178 |

## Chapter 5

**Ecological Aspects of Heavy Metal Responses in Microorganisms**

Trevor Duxbury

|  |     |
|--|-----|
| 1. Introduction  | 185 |
| 2. Effects of Heavy Metals on Microbial Communities          | 187 |
| 2.1. Abundance   | 187 |
| 2.2. Diversity   | 188 |
| 2.3. Metal Tolerance   | 191 |
| 2.4. Evolution and Gene Transfer                             | 196 |
| 3. Effects of Heavy Metals on Microbially Mediated Processes | 199 |
| 3.1. Carbon Fixation   | 199 |
| 3.2. Methanogenesis  | 200 |
| 3.3. Respiration   | 201 |
| 3.4. Litter Decomposition                                    | 201 |
| 3.5. Dinitrogen Fixation                                     | 203 |
| 3.6. Other Nitrogen Transformations                          | 205 |
| 4. Effects of Heavy Metals on Various Interactions           | 205 |
| 4.1. Substrate Interactions                                  | 206 |

|  |     |
|--|-----|
| 4.2. Predator-Prey Interactions .....  | 209 |
| 4.3. Interfacial Interactions .....    | 212 |
| 4.4. Pathogenic Interactions .....     | 213 |
| 5. Common Problems .....               | 216 |
| 5.1. Field-Oriented Studies .....      | 216 |
| 5.2. Laboratory-Oriented Studies ..... | 219 |
| 5.3. Problems in General .....         | 222 |
| 6. Future Prospects .....              | 227 |
| References .....                       | 228 |

## Chapter 6

### Ecology of Microbial Cellulose Degradation

Lars G. Ljungdahl and Karl-Erik Eriksson

|   |     |
|---|-----|
| 1. Introduction .....   | 237 |
| 2. Structure of Cellulose .....                                     | 238 |
| 3. Cellulolytic Microorganisms .....                                | 242 |
| 3.1. Properties of Wood-Rotting Fungi .....                         | 244 |
| 3.2. Anaerobic Cellulolytic Fungi .....                             | 246 |
| 3.3. Cellulolytic Enzyme Systems in Fungi .....                     | 247 |
| 3.4. Cellulolytic Bacteria and Their Cellulase Enzyme Systems ..... | 253 |
| 4. Interaction among Microorganisms in Ecological Systems .....     | 270 |
| 4.1. Cellulose in Soils .....                                       | 271 |
| 4.2. Cellulose Degradation in an Aerobic Environment .....          | 272 |
| 4.3. Cellulose Degradation in an Anaerobic Environment .....        | 273 |
| 5. Conclusion .....   | 278 |
| References .....  | 279 |

|             |     |
|-------------|-----|
| Index ..... | 301 |
|-------------|-----|

# Microbial Interactions among Aerobic and Anaerobic Sulfur-Oxidizing Bacteria

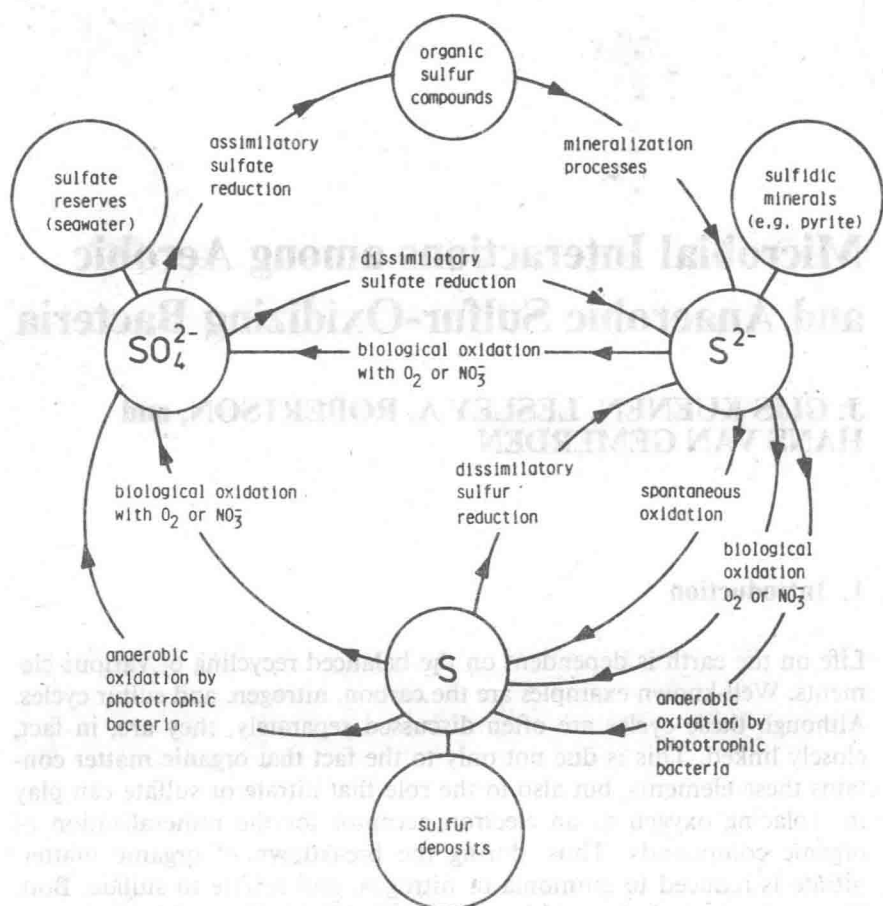
J. GIJS KUENEN, LESLEY A. ROBERTSON, and  
HANS VAN GEMERDEN

## 1. Introduction

Life on the earth is dependent on the balanced recycling of various elements. Well-known examples are the carbon, nitrogen, and sulfur cycles. Although these cycles are often discussed separately, they are, in fact, closely linked. This is due not only to the fact that organic matter contains these elements, but also to the role that nitrate or sulfate can play in replacing oxygen as an electron acceptor for the mineralization of organic compounds. Thus, during the breakdown of organic matter, nitrate is reduced to ammonia or nitrogen, and sulfate to sulfide. Both the ammonia and the sulfide can be reoxidized. Since this chapter is mainly concerned with the ecology of bacteria involved in the sulfur cycle, a brief discussion of this cycle is appropriate (Pfennig and Widdel, 1982; Kuenen, 1975; Trudinger, 1982). Sulfate serves as the sulfur source for the biosynthesis of organic sulfur compounds by plants and microorganisms using the process known as assimilatory sulfate reduction (Fig. 1). In biological materials, sulfur is usually present in its most reduced form (e.g., as sulfide in amino acids such as cysteine). During the decomposition of this material under aerobic conditions, the organic sulfide is initially oxidized and subsequently released as sulfate. Under anaerobic

---

J. GIJS KUENEN and LESLEY A. ROBERTSON • Laboratorium voor Microbiologie, Delft University of Technology, Delft, The Netherlands. HANS VAN GEMERDEN • Laboratorium voor Microbiologie, University of Groningen, Haren, The Netherlands.



**Figure 1.** The sulfur cycle. [Adapted from Bos and Kuenen (1983).]

conditions, the sulfide is liberated as hydrogen sulfide. Another important source of hydrogen sulfide is from dissimilatory sulfate reduction, whereby sulfate-reducing bacteria can use sulfate as their electron acceptor for the oxidation of organic compounds or molecular hydrogen. Some sulfide precipitates as ferrous sulfide or forms pyrite, but much of it is reoxidized via elemental sulfur to sulfate (Bos and Kuenen, 1983). This can be done under anaerobic conditions in the light by phototrophic sulfur-oxidizing bacteria, which use the sulfide as an electron donor in the generation of reducing power for the reduction and assimilation of carbon dioxide (Trüper and Fischer, 1982). By this process, the electrons produced in mineralization are rechanneled into organic compounds.

Elemental sulfur, which may be formed as an intermediate, can also be reduced to sulfide by sulfur-reducing heterotrophs. Under aerobic or denitrifying conditions hydrogen sulfide can also be spontaneously oxidized by oxygen or nitrate if these substances interact at high enough concentrations. At lower concentrations of oxygen (or nitrate), sulfide is usually oxidized by the colorless sulfur bacteria to give sulfate (Kelly, 1982). During this process, the majority of the electrons from the sulfide are used to reduce oxygen or nitrate to give water or molecular nitrogen, respectively. Some electrons can be used for  $\text{CO}_2$  reduction and be recycled into organic compounds. Thus, it is the complementary action of the two types of oxidative bacteria with the sulfur and sulfate-reducing bacteria that maintains most of the global sulfur cycle. However, it should be remembered that industrial pollution and geothermal processes also contribute substantially.

Both the phototrophic and colorless sulfur bacteria comprise large, heterogeneous groups of organisms. Whereas the difference between the two is obviously based on the possession or lack of photosynthetic pigments, the groups of different species can be further subdivided by their degree of physiological specialization or versatility, type of photosynthetic pigmentation, and other characteristics, for example, the ability to denitrify. These subdivisions are dealt with in Section 2, and are summarized in Tables I and II.

The sulfide-oxidizing bacteria are dependent on reduced sulfur compounds for growth, and therefore are found in environments where sulfate reduction occurs or where a geological source of sulfur compounds is available. Since most of the colorless sulfur bacteria are dependent on oxygen, they often live at the interface between aerobic and anaerobic zones where low concentrations of oxygen and sulfide can coexist. Examples of such environments are the aerobic surfaces of otherwise anaerobic freshwater marine sediments and the interface between the aerobic and anaerobic zones of stratified bodies of water. An example of such an interface is found in Solar Lake (Sinai), where sulfide and oxygen coexist over a depth of a few centimeters (Fig. 2). In sediments, this layer can be as narrow as 0.1 mm or less (Jørgensen, 1982). Under special circumstances, blooms of colorless sulfur bacteria (e.g., *Beggiatoa* mats) can be found on the surface of anaerobic sediments. When light can reach these interfaces, the phototrophic sulfur bacteria may thrive, and in many stratified lakes annual blooms of a variety of these phototrophs occur. These blooms can produce intensely colored green, brown, or red layers sometimes as thick as 1 m. Blooms of these organisms can also be found in the top layers of light-exposed sediments, where they can, for example, form a discrete layer in the complex microbial communities of algal mats.

In our laboratories we have been investigating the occurrence and

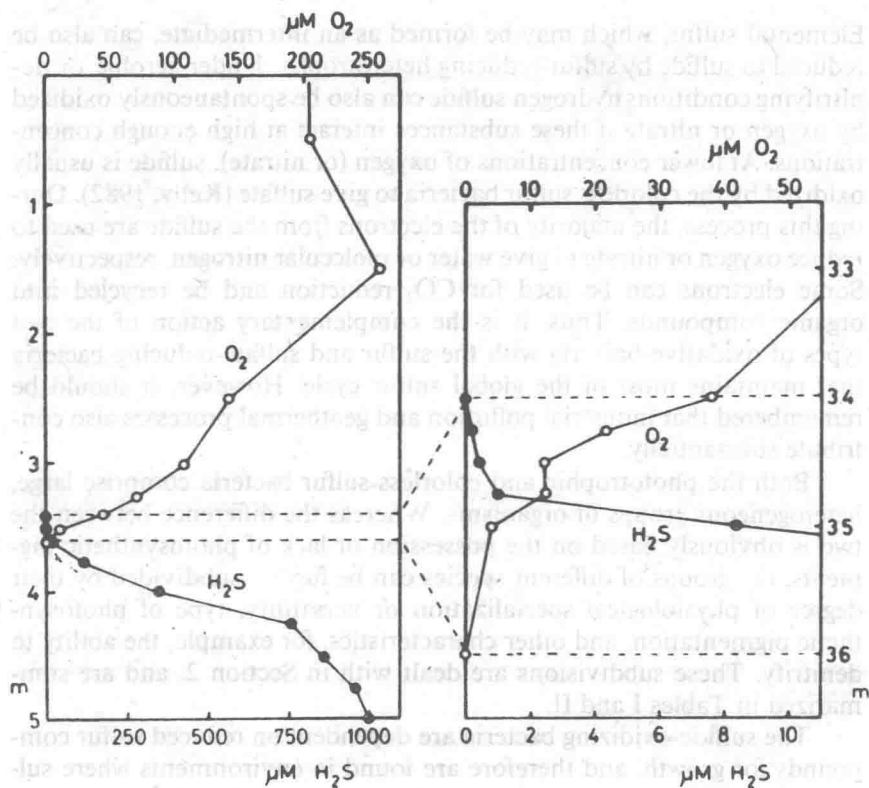


Figure 2. Concentrations of sulfide and oxygen in Solar Lake (Sinai) as a function of depth. Oxygen and sulfide can be seen to coexist at the interface, [Jørgensen *et al.* (1979).]

ecological niches of the seemingly endless variety of species among the colorless and phototrophic bacteria. In addressing these questions, we have tried to consider the abiotic environmental parameters together with some of the biotic variables, especially the interactions that occur among the phototrophic and colorless sulfur bacteria as well as between the two groups. Our approach has been through an ecophysiological study of pure cultures of these organisms under conditions that may be relevant to their existence in nature. As a second step, we have studied mixtures of the pure cultures of these organisms, and in a few cases we have also done some field studies to verify predictions made in the laboratory.

One of the most important environmental pressures imposed on microorganisms is that of nutrient or light limitation. An understanding of bacterial response to these limitations and their survival under such conditions is crucial to a better knowledge of microbial ecology. For the



study of the ecophysiology and interactions of microorganisms under nutrient limitation in the laboratory, continuous cultivation in a chemostat has been an indispensable technique. In our investigation of the interactions between the different types of sulfur bacteria, this technique has allowed us to study the physiology of test organisms under a variety of nutrient limitations, and also to use the chemostat as a device by which the selective pressures exerted on species competing for limiting nutrients or light can be simulated and amplified.

In Section 4 we describe a selected number of examples of microbial competition involving the sulfur-oxidizing bacteria. It will become clear that many of the phototrophs and the colorless sulfur bacteria are very well suited for use as model organisms for the exploration of the basic principles that determine the survival value of different metabolic strategies in the struggle for existence.

## 2. The Types of Bacteria

### 2.1. The Colorless Sulfur Bacteria

The group of organisms known as the colorless sulfur bacteria make up a heterogeneous collection of Gram-negative bacteria, which includes intensively studied species, such as some members of the genus *Thiobacillus*, and others that have not been obtained in pure culture and have only been studied superficially, such as the genus *Thiobacterium* (Vishniac, 1974; la Rivière, 1974). The various physiological types represented within the group are shown in Table I. Colorless sulfur bacteria are found at a wide range of temperatures, pH values, and degrees of aerobiosis or anaerobiosis. Some are obligate chemolithoautotrophs (e.g., *T. neapolitanus*), some can only oxidize sulfur compounds if they are supplied with an organic carbon source (e.g., *T. perometabolis*), while others are capable of autotrophic, heterotrophic, or mixotrophic growth (e.g., *T. novellus*). The group is commonly subdivided on the basis of the degree of physiological specialization shown by the various species (Tables I and III).

#### 2.1.1. The Obligate Chemolithoautotrophs

These highly specialized species can only grow autotrophically. They must use an inorganic source of energy and obtain cell carbon from carbon dioxide fixation via the Calvin cycle. Most, however, are able to utilize small amounts of exogenous organic carbon (Matin, 1978), which can serve as a source of carbon, but not energy. The citric acid cycle in these organisms is inoperative, its enzymes only serving for biosynthesis. With