BIOLOGY OF ANAEROBIC MICROORGANISMS

Edited by Alexander J. B. Zehnder

Wiley Series in Ecological and Applied Microbiology
Series Editor: Ralph Mitchell

Biology of Anaerobic Microorganisms

edited by

ALEXANDER J. B. ZEHNDER
Agricultural University
Wageningen, The Netherlands

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Alexander J. B. Zehnder Editor

CONTRIBUTORS

JAN AMESZ Department of Biophysics, Huygens Laboratory, University of Leiden, Wassenaarseweg 78, 2300 RA Leiden, The Netherlands

PATRICIA J. COLBERG Western Research Institute, P.O. Box 3395, University Station, Laramie, WY 82071, USA

JAN DOLFING Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG), CH-6047, Kastanienbaum, Switzerland

GUY FAUQUE ARBS-Equipe Commune d'Enzymologie, CNRS-CEA, CEN-Cadarache, F-13108-Saint-Paul-Lez-Durance, Cédex, France

WILLIAM C. GHIORSE Department of Microbiology, Cornell University, Ithaca, NY 14853, USA

JAN T. KELTJENS Department of Microbiology, Faculty of Science, University of Nijmegen, Toernooiveld, NL-6525 ED, Nijmegen, The Netherlands

DAVID B. KNAFF Department of Chemistry, Texas Tech University, Lubbock, TX 79409, USA

JEAN LEGALL Biochemistry Department, University of Georgia, Athens, GA 30602, USA

MICHAEL J. MCINERNEY Department of Botany and Microbiology, University of Oklahoma, 770 Van Vleet Oval, Norman, OK 73019, USA

MICHAEL T. MADIGAN Department of Microbiology, Southern Illinois University, Carbondale, IL 62901, USA

RONALD S. OREMLAND U.S. Geological Survey, 345 Middlefield Road, Menlo Park, CA 94025, USA

Bernhard Schink Lehrstuhl Mikrobiologie I, Eberhard-Karls-Universität, Auf der Morgenstelle 28, D-7400 Tübingen, Federal Republic of Germany

Adriaan H. Stouthamer Biological Laboratory, Vrije Universiteit, Postbus 7161, 1007 MC, Amsterdam, The Netherlands

Werner Stumm Swiss Federal Institute of Technology, Zürich Institute for Water Resources and Water Pollution Control (EAWAG), CH-8600 Dübendorf, Switzerland

James M. Tiedje Departments of Crop and Soil Sciences and of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824, USA

Chris van der Drift Department of Microbiology, Faculty of Science, University of Nijmegen, Toernooiveld, NL-6525 ED, Nijmegen, The Netherlands

Godfried D. Vogels Department of Microbiology, Faculty of Science, University of Nijmegen, Toernooiveld, NL-6525 ED, Nijmegen, The Netherlands

Friedrich Widdel Mikrobiologie, Fachbereich Biologie, Philipps-Universität, D-3550 Marburg, Federal Republic of Germany

Alexander J. B. Zehnder Department of Microbiology, Agricultural University, Hesselink van Suchtelenweg 4, NL-6703 CT Wageningen, The Netherlands

SERIES PREFACE

The Ecological and Applied Microbiology series of monographs and edited volumes is being produced to facilitate the exchange of information relating to the microbiology of specific habitats, biochemical processes of importance in microbial ecology, and evolutionary microbiology. The series will also publish texts in applied microbiology, including biotechnology, medicine, and engineering, and will include such diverse subjects as the biology of anaerobes and thermophiles, paleomicrobiology, and the importance of biofilms in process engineering.

During the past decade we have seen dramatic advances in the study of microbial ecology. It is gratifying that today's microbial ecologists not only cooperate with colleagues in other disciplines but also study the comparative biology of different habitats. Modern microbial ecologists, investigating ecosystems, gain insights into previously unknown biochemical processes, comparative ecology, and evolutionary theory. They also isolate new microorganisms with application to medicine, industry, and agriculture.

Applied microbiology has also undergone a revolution in the past decade. The field of industrial microbiology has been transformed by new techniques in molecular genetics. Because of these advances, we now have the potential to utilize microorganisms for industrial processes in ways microbiologists could not have imagined 20 years ago. At the same time, we face the challenge of determining the consequences of releasing genetically engineered microorganisms into the natural environment.

New concepts and methods to study this extraordinary range of exciting problems in microbiology are now available. Young microbiologists are increasingly being trained in ecological theory, mathematics, biochemistry, and genetics. Barriers between the disciplines essential to the study of modern microbiology are disappearing. It is my hope that this series in Ecological and Applied Microbiology will facilitate the reintegration of microbiology and stimulate research in the tradition of Louis Pasteur.

Anoxic environments are widespread in the biosphere, yet we understand very little about the biological processes occurring in anoxic habitats. In recent years new insights have been gained into the physiology, genetics, and ecological function of anaerobic microorganisms. Alexander Zehnder has been in the forefront of this effort. In this volume he has invited contributions from researchers working in a wide range of disciplines related to the biology of anaerobes. It is my hope that, through the contributions in this volume, readers will gain a better understanding of anaerobic microbial processes and will be stimulated to attempt to answer some of the many unsolved questions about the activities of anaerobes.

RALPH MITCHELL

Cambridge, Massachusetts
October 1987

PREFACE

Anaerobic microorganisms attracted the curiosity of scientists over 100 years ago because it was noted that many of these organisms either improved or spoiled food and drink, or could convert waste into methane. Since the beginning of the twentieth century, an increasing number of anaerobes have been applied to the production of fine and bulk chemicals in industry. In the 1950s and 1960s the interest of most general microbiologists switched from classical fermentation research to the new field of molecular biology, and few remained interested in anaerobic microorganisms and processes. These researchers formed a then small group in which everybody knew everybody. One of the consequences of the energy crisis in 1973 was that many scientists rediscovered the anaerobes and their potential, and research developed around methanogenic and photosynthetic bacteria. At about the same time the growing concern of people about the environment initiated a discussion of the impact of intensive agricultural practice on fertile soil and water quality, thus stimulating research on denitrification. The improvement of cultivation techniques for anaerobes and the discovery of archaebacteria as a new phylogenetic kingdom through study of the 16S rRNA of methanogens led to a burst of research activities with anaerobic microorganisms. Progress in this field is now very rapid and the small group of scientists dealing with anaerobes has become a good-sized community that is steadily growing.

The idea to write a book on fundamental and applied aspects of anaerobes was born in one of many late-night discussions during the First Advanced Course of Microbial Ecology in Kastanienbaum, Switzerland. We mutually agreed that a book on the great diversity of molecular, ecological, and applied aspects of this fascinating group of microorganisms was absolutely needed and we were all convinced that half the work had already been accomplished by the dawn of the new day. But as usually happens with such intentions, the project was put on ice and not pursued further. However, when asked some time later by Trev Leger to edit this book, my initial enthusiasm returned immediately. Luckily, many of the leading scientists (including some from the night-long discussion) who are actively studying various aspects of anaerobic microorganisms became enthusiastic and agreed to contribute chapters to the book. Unfortunately, because of various problems, the book could not be published earlier. I would like to thank all the authors who were on time with their manuscripts for their patience.

It is my hope that the book will not only be useful for those engaged in research with anaerobes and anaerobic processes, but that it will act as a catalyst for students to dedicate themselves to this promising field with its enormous potential for the future.

ALEXANDER J. B. ZEHNDER

Wageningen, The Netherlands
June 1988

Biology of Anaerobic Microorganisms

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GEOCHEMISTRY AND BIOGEOCHEMISTRY OF ANAEROBIC HABITATS

ALEXANDER J. B. ZEHNDER

Department of Microbiology, Agricultural University, Hesselink van Suchtelenweg 4, NL-6703 CT Wageningen, The Netherlands

WERNER STUMM

Swiss Federal Institute of Technology, Zürich, Institute for Water Resources and Water Pollution Control, CH-8600 Dübendorf, Switzerland

1.1 INTRODUCTION

On a global average, the environment is, with regard to a proton and electron balance, in a stationary situation corresponding to a present-day atmosphere of 20.9% O_2, 0.03% CO_2, 79.1% N_2, a world ocean with pH $\approx$ 8 and a redox potential of $E_H = 0.75$ V. This situation is the result of an interplay of different types of reactions, namely geochemical and biochemical processes.

The geochemical processes can best be understood considering the schematic reaction proposed by Sillén (32):

$$\text{igneous rock} + \text{volatile substances} \longleftrightarrow \text{air} + \text{seawater} + \text{sediments} \quad (1)$$

According to this equation, the volatiles (H_2O, CO_2, N_2, HCl, HF, SO_2, CH_4, H_2S, H_2, NH_3) that have leaked from the interior of the earth through different "cracks" (volcaneous, volcanic activities at the oceanic ridges, etc.) have reacted in a giant acid-base and redox reaction with the rocks (silicates, oxides, and carbonates). The result of what is called weathering was a certain kind of atmosphere, seawater, and sediment. Their composition is, over geological time, still changing slightly as a function of volcanic activities. If these activities were to cease, the surface of the earth would reach an equilibrium and all processes would stop. In other words, the earth would be geochemically dead.

However, photosynthesis, quantitatively and qualitatively the most important biochemical process on earth, can be regarded as a local and time-limited reversal in the universal drift toward equilibrium. Photosynthetic reactions capture energy from solar radiation, and photosynthesis may be perceived as a process producing localized centers of highly reduced redox intensities (e.g., organic molecules) and a reservoir of oxygen. The nonphotosynthetic organisms tend to restore the equilibrium through energy-yielding redox reactions (Fig. 1.1). Only the steady shift away from chemical equilibrium as a result of photosynthesis allowed the biosphere to develop and in turn to influence the composition of its environment (i.e., atmosphere, soil, water, and sediments) (20). The distribution and the redox state of the elements in the present-day environment is thus the result of a very sensitive overall balance between a process driven by the chemical heterogeneity of our planet and the impact of solar energy.

On a global average the oxidation states of weathering sources and the oxygen from photosynthesis equal those of soil and sedimentary products. However, the balance might, locally and temporarily, be significantly upset due to the hetero-

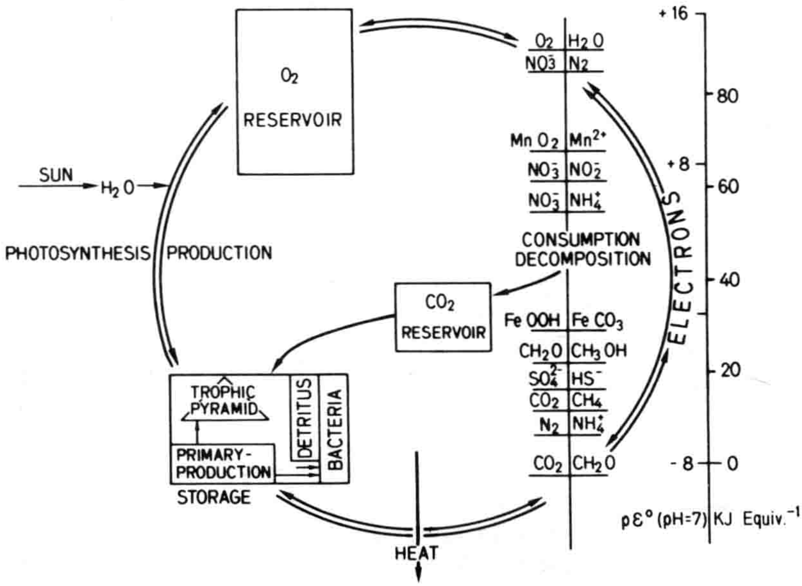

Figure 1.1 Photosynthesis and biochemical cycle. Photosynthesis may be interpreted as a disproportion into an oxygen reservoir and reduced organic matter (biomass containing high-energy bonds made with hydrogen and carbon, nitrogen, sulfur, and phosphorus compounds). The nonphotosynthetic organisms tend to restore equilibrium by catalytically decomposing the unstable products of photosynthesis through energy-yielding redox reactions. The scale of redox couples on the right gives the sequence of the redox reactions observed in an aqueous system. From Stumm and Morgan (35).

geneity of the environment. This heterogeneity can be amplified by various factors, among which the kinetic factors are quantitatively the most important. In well-aerated soils, for example, micro-environments can be found where no oxygen is present and thus anaerobic processes can take place. On a macroscopic scale such a situation should not exist since oxygen abounds. But as a result of the fast rate of oxygen consumption by the biota oxidizing organic material and the slow diffusion of oxygen to the place of its consumption, microscopically and temporarily a situation may occur which is not in equilibrium with the macroscopic (global) environment. Other, larger and more stable anaerobic environments can develop in various sediments. These anaerobic habitats are the result of two processes: (a) the access of oxygen to the sediments is considerably hindered by the water overlaying them because of the low solubility of oxygen in water and the slow diffusion of oxygen within the water body; and (b) part of the photosynthetic organic material produced in the photic zone reaches the sediments far more quickly than does the oxygen. As a consequence of the biological degradation processes, oxygen is depleted within a short time in the surface zones of the sediments. The depth of the oxygenated zone is primarily a function of the input rate of easily degradable