



# Microbes and the Environment

Perspectives and Challenges

(微生物与环境：前景与挑战)

*Edited by*

Shuang-Jiang Liu

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Beijing

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# **Microbes and the Environment: Perspectives and Challenges**

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## Preface

A group of microbiologists from China, Germany, and additional European countries met in Beijing in September 2007 and held a conference entitled “Microbes and the Environment”. The purpose of this conference was to evaluate how microbes (a) shape the planet and (b) might be harnessed to solve environmental problems. This book provides an overview of many of the contributions made during this conference.

We gratefully acknowledge sponsors that provided support for this conference, including the China-Germany Scientific Center [a center jointly funded by the Deutsche Forschungsgemeinschaft (DFG) and National Natural Science Foundation of China (NSFC)], the Chinese Society for Microbiology, the Institute of Microbiology at Chinese Academy of Sciences, and the University of Bayreuth. We are likewise indebted to Yuan Li and Chengying Jiang for excellent assistance in formatting this monograph, as well as overseeing many organizational matters during the conference.

Lastly, we extend a special thanks to the contributing authors of this monograph and hope that it will provide an impetus for the next joint effort to evaluate the environmental importance of microorganisms.

*Shuang-Jiang Liu and Harold L. Drake*  
*Beijing and Bayreuth, March 2008*

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# **SECTION A**

## **INTRODUCTION**



# **CHAPTER 1**

## **CURRENT AND FUTURE PERSPECTIVES ON THE ENVIRONMENTAL IMPORTANCE OF MICROORGANISMS**

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### **INTRODUCTION**

In contrast to plants and animals that paint our landscapes and oceans with fascinating shapes and vivid colors, most microorganisms, especially prokaryotic microorganisms, are not easily seen. Thus, it is primarily the effect that the microbe has on its habitat, rather than the visual image of the microbe itself, that makes its presence known to humans. Indeed, the impact of microbes on human health and the spoilage and production of foods are primary reasons why microbiology became a scientific discipline (Brock, 1961). Thus, many of the primary roots of microbiology are hinged to human health and diet, as is exemplified by the emergence of so-called ‘probiotics’ as dietary supplements, and a societal awareness of the impact of microbes on the environment has lagged behind the more practical appreciation of what microbes are relative to the human experience. Nonetheless, Winogradsky (1890), Beijerinck (1908), Kluyver (1924), and other early microbiologists understood that the metabolic diversity of microorganisms was important to biological cycling of the elements. It is now clear that microbes have enormous impact on the global flux of elements, as is illustrated by the fact that, at the global level, the amount of carbon and nitrogen stored in microbial biomass is 1- and 10-fold that of plants, respectively (Whitman et al., 1998).

It is within this context, i.e., the context of the importance of the microbe in the environment, that a group of scientists from China and Europe met in September 2007 to evaluate many of the key issues facing not only microbiologists as scientists, but also society relative to how microbes shape the planet. The purpose of this introductory chapter is to highlight both current and future perspectives on the environmental importance of microorganisms that were evaluated during this meeting. Subsequent chapters provide detailed statements on both basic and applied problems that were identified during the course of this meeting.

### **MICROBIAL CYCLING OF ELEMENTS IN DIVERSE HABITATS**

**Intercycle coupling** Microbe-catalyzed redox reactions fuse the biological cycles.

Thus, one of the main impacts that microbes have in the environment is ‘intercycle coupling’ (Drake and Küsel, 2005; Drake et al., 2008). For example, sulfate reducers fuse the sulfur and carbon cycles during the concomitant dissimilation of oxidized sulfur and the oxidation of reduced (i.e., organic) carbon, and denitrifiers fuse the nitrogen and carbon cycles during the concomitant dissimilation of nitrate and the oxidation of reduced (i.e., organic) carbon. At the cellular level, the conservation of energy is the main incentive for the microbe to form links between diverse half-cell reactions that, collectively, constitute biological cycles. But at the ecosystem level, these redox links manifest themselves in ways that have profound impact on global cycling of elements (e.g., C, N, and S). Indeed, it is textbook knowledge that many of the reactions of the biological cycles are catalyzed exclusively by microbes (e.g., Madigan and Martinko, 2006), which obviously make microorganisms indispensable to these cycles as we know them. It is likewise textbook knowledge that the large number of unique microbial links in the biological cycles is coincident to and dependent on microbial biodiversity.

**Biodiversity** Twenty years ago, the term ‘biodiversity’ was not an entry in the Third College Edition of the Webster’s New World Dictionary. Although the term is now in widespread use, the general populace might think of ‘biodiversity’ as the abundance of different plants and animals within a particular region. Such thinking of course overlooks the unseen microbe, but more importantly fails to take into consideration that biodiversity has two components, one being organismal and the other being functional. Thus, the enormous phylogenetic diversity of microorganisms that has become a hallmark of microbial ecology in recent years masks an enormous physiological diversity that constitutes the primary basis for the *in situ* functional links of microbes and, thus, their environmental importance. Numerous studies have shown that nature harbors an enormous number of undiscovered microbes due to the evolutionary driving forces that have yielded these complementary (i.e., phylogenetic and functional) diversities. For example, the major fraction of the estimated  $10^6$  distinct prokaryotic genomes (i.e., species) in soil is unresolved (Curtis et al., 2002; Gans et al., 2005).

**Molecular microbial ecology** The application of molecular approaches has unquestionably become the modern ‘high card’ of microbial ecology. Indeed, progress in the field of molecular microbial ecology has issued in a new era of not only how microbes can be analyzed but also how we think of microbes relative to their phylogenetic diversity and *in situ* realities. It is nonetheless worth noting that both classic and improved cultivation approaches can also detect a broad diversity of taxa that may have once been considered ‘unculturable’ and also provide direct information as to their function and regulation (e.g., Jansen et al., 2002; Joseph et al., 2003; Stevenson et al., 2004; Jansen, 2006; Heylen et al., 2006). Indeed, in certain cases, the detection of *in situ* relevant functional groups by cultivation-based methods might be superior to molecular ones (e.g., Ellis et al., 2003; Macur et al., 2007). Nonetheless, all methods have limits.

Thus, there is a growing awareness that a combination of both classical and molecu-

lar approaches is needed in 'modern' microbial ecology. For example, cataloging molecular sequences is important for resolving certain types of phylogenic-based questions but may not replace the type of information obtained from cultured organisms relative to their ecophysiological behavior and how this behavior impacts on ecosystem function. Indeed, 16S rRNA-based phylogenetic analyses have serious shortcomings relative to establishing functional links to many microbial taxa (e.g., Becker et al., 2000; Jansen, 2006; von Wintzingerode et al., 1997; Phillipot, 2002; Hallin et al., 2006; Drake et al., 2006).

**Postulates of the past: do we need them?** As noted by Hungate in the early 1960's (Hungate, 1962), one of the main goals in the field of microbial ecology is to determine what impact a particular microbe has on the system it inhabits, i.e., to resolve cause-and-effect relationships of environmentally important microorganisms. Principles for establishing a cause-and-effect relationship between a microorganism and a disease (i.e., its *in situ* activity) were documented by Koch in the 1880's (Koch, 1882, 1884), and several postulates attributed to Koch can be used to prove that a specific microbe causes a specific disease. Disease is merely one of the many symbioses of microbes, and it is therefore possible to slightly modify Koch's postulates for evaluating the ecological activity (i.e., effect or impact) of a microbe:

- (1) A specific microbe must be associated with an *in situ* activity of a specific habitat.
- (2) This microbe must be isolated in pure form from this habitat.
- (3) This microbe must produce the same activity observed in the habitat when challenged under experimental conditions (e.g., in a microcosm or bioreactor) that simulate those of the habitat from which it was isolated.
- (4) This microbe must be re-isolated from the experiment (e.g., from the microcosm or bioreactor) conducted in Postulate 3.

The current awareness of the so-called 'uncultured majority' greatly complicates the implementation of these postulates, i.e., the inability to culture a microbe and the difficulty in accurately simulating *in situ* conditions in the laboratory obviously limit the utility of these postulates. The large number phylotypes likewise augment the difficulties one faces with complex communities such as soil microbial biomes. Although these postulates might be considered a 'classic wish list' relative to proving cause-and-effect relationships of microbes in their given habitats, the difficulties noted above clearly point to the need to employ non-classic (i.e., molecular) methods and to modify these postulates accordingly when the question posed so necessitates.

Many chapters in this monograph evaluate environmental aspects of functional groups of microorganisms and confront key issues relative to resolving their *in situ* activities. As outlined in the chapters, and depending of the problem under study, these issues can be addressed by both cultivation-dependent and cultivation-independent approaches, each of which presents its own unique strengths and weaknesses relative to fulfilling the postulates above. One of the future challenges facing microbiologists is to

work objectively within the constraints of methods that are over shadowed by well-established postulates and principals of the past.

## BIOREMEDIATION AND BIOAUGMENTATION: HARNESSING MICROBES

**Microorganisms: the catalyst on center stage** Microorganisms are the main catalysts in many bioremediation and bioaugmentation processes. Microbiologists often claim that the efficient exploitation of microorganisms needs a clear understanding and complete description of the microbes. This claim is certainly true for many current industrial bioprocesses, such as the fermentative productions of antibiotics and amino acids. These commercial processes exploit pure microbial cultures, such as *Streptomyces coelicolor* or *Corynebacterium glutamicum*. However, is this claim also true for bioremediation and bioaugmentation technologies, particularly those in the early days of exploiting microorganisms before their existence was scientifically established? The Chinese invented the use of spoiled water for the recovery of copper from ores as early as 1094, which could be considering the beginning of bioleaching (Zhong, 1980). Thus, bioleaching was established prior to a scientific understanding of bioleaching microbes such as *Thiobacillus ferrooxidans* (Temple and Colmer, 1951) [currently *Acidithiobacillus ferrooxidans* (Kelly and Wood, 2000)]. The Imhoff (also called Emscher-Brunnen) tank, which separates and digests organic particles in sewage, was invented prior to understanding that anaerobic digestion is driven by anaerobic bacteria (Winter, 1999). That the majority of microbes in activated sludge are still uncharacterized (Amann et al., 1995) has not prevented the operation of countless wastewater treatment plants. Thus, a sophisticated understanding of microorganisms might appear to not be prerequisite to harnessing them for bioremediation and bioaugmentation. But, is this conclusion fully correct?

Although wastewater treatment plants might operate relatively effectively, their efficiency and stability are adversely affected when activated sludge increases abnormally. This phenomenon is called sludge bulking and is attributed to an abnormal growth of filamentous microbes in activated sludge (Schuler and Jassby, 2007). Microbiologists and municipal engineers are still looking for ways to hinder the growth of filamentous microbes in activated sludge. A fundamental question that needs to be answered is: If the instability of the overall biological process is caused by microbes at a specific position of the process, what *in situ* factors and what aspects of the problematic microbes govern this instability? Such fundamental questions make it clear that more studies are needed to understand (a) the microbial composition of activated sludge and (b) the functionality of specific microbes involved in the removal of pollutants in wastewater.

An understanding of the microbes involved in bioremediation and bioaugmentation is not only important for improving current environmental biotechnologies, but also vital for developing novel future biotechnologies. For example, the discovery of anaerobic



ammonium oxidizers led to the application of a novel process (i.e., Anammox) for the removal of nitrogen in wastewater (Jetten et al., 2005). It is textbook knowledge that microbes are major drivers of the geobiochemical cycles on earth. It can be assumed that bioremediation or bioaugmentation processes are anthropogenic-induced augmentations of microbial reactions/metabolisms. It thus seems obvious that understanding the fundamentals of naturally-occurring microbial processes is vital for the efficient augmentation of microbes in novel environmental technologies.

**Microbial conversion of metals** Many microbes can oxidize and reduce metals. This capacity (a) has been exploited in the biomining and bioleaching (i.e., for the recovery) of valuable metals (such as copper and gold) from low-grade ores (Rawlings and Johnson, 2007) and (b) is important for the bioremediation of acid mine drainage-impacted environments (Saria et al., 2006). In natural environments such as geothermal regions and in lake sediments, reduced sulfur (e.g., sulfide) often coexists with metals and can be simultaneously oxidized by different members of the microbial community, including iron- and sulfur-oxidizers. The microbial oxidation of iron and sulfur is a major driver of elemental geocycles in geothermal regions and in lake sediments (Inskeep et al., 2004; Macur et al., 2004; Kozubal et al., 2007; Küsel et al., 2002; Blöthe et al., 2008).

Sulfate is the most oxidized sulfur compound, and sulfate-reducing bacteria have been long recognized as an important group of anaerobes that reduce sulfate to sulfide as a terminal electron-accepting process (Postgate, 1978; Thauer et al., 2007). Sulfate reducers are notorious for producing unpleasant odors and are primary contributors to the biological corrosion of steel (Postgate, 1978; Zuo, 2007). In contrast to such annoyances, sulfate reducers can also be exploited for the biological treatment of, and metal recovery from, wastewater that contains high concentrations of sulfate and metal ions (e.g., Lens et al., 2007; Liamleam and Annachatre, 2007).

**Microbial conversions of aromatic compounds and persistent organic pollutants (POPs)** Although the scope of environmental microbiology has become very broad, one of its most important aspects is rooted to the environmental fate of pollutants. Investigations on the environmental fate of BTX (benzene, toluene, and xylene) and other aromatic compounds have yielded a basic understanding of how industrial and agricultural pollutants are degraded by microbial communities (Golovleva et al., 1992; Chaudhry and Chapalamadugu, 1991; Osborne et al., 1988; Johnson and Spain, 2003). Although recently synthesized chlorinated nitroaromatic compounds (e.g., chloronitrobenzenes) are likely to be resistant to microbial attack, progress on how microbes might be utilized to degrade such compounds from impacted sites is being made (Wu et al., 2006; Liu et al., 2007). The discovery of many novel biocatalysts and biochemical reactions during research on the microbial degradation of aromatic compounds has been a new impetus in the field of microbial physiology. Genomics and metagenomics provide new strategies to acquire information of relevance in environmental microbiology: instead of first identifying microbial phenotypes pertinent