TECHNIQUES FOR THE ASSESSMENT OF MICROBIAL PRODUCTION AND DECOMPOSITION IN FRESH WATERS

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Techniques for the Assessment of Microbial Production and Decomposition in Fresh Waters

Edited by

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Foreword

This is the sixth handbook to be issued by Section PF (Production in Freshwaters) of the IBP.*

Previous handbooks are: *Handbook No.* 3—Methods for Assessment of Fish Production in Freshwaters—edited by W. E. Ricker (1968), now appearing in a considerably revised edition (1971). *Handbook No.* 8—Methods for Chemical Analysis of Freshwaters—edited by H. Golterman with the assistance of R. S. Clymo (1968, 1970, 1971). *Handbook No.* 12—A Manual of Methods of Measuring Primary Productivity in Aquatic Environments—edited by R. A. Vollenweider (1969, 1971). *Handbook No.* 17—A Manual of Methods for the Assessment of Secondary Productivity in Freshwaters—edited by W. T. Edmondson and G. G. Winberg (1971). *Handbook No.* 21—Project Aqua, A Source Book of Inland Waters Proposed for Conservation—edited by H. Luther and J. Rzóska (1971).

The present volume is an attempt to bring together the main methods of microbial assessment. The role of micro-organisms in the biological functioning of a water body is fundamental through chemo- and photo-synthesis and decomposition and has a profound effect on the circulation of nutrients. Their role was recognized a long time ago but only recently was defined with some precision. Without the assessment of the role of microbial organisms, the complexity of production cannot be grasped fully. It is, therefore, gratifying that, within the IBP, an attempt has been made to collect and present the available methods of research, even though some of them have not yet reached finality.

This was a difficult task undertaken by a number of microbiologists from eight countries during a working meeting in Leningrad (1969) and subsequently by meetings of the editors—Dr. Y. Sorokin, chief biologist at the Institute of Inland Waters of the U.S.S.R. Academy of Sciences at Borok; Professor H. Kadota, working at the Research Institute for Food Science at

* The International Biological Programme is a worldwide plan concerned with 'the biological basis of productivity and human welfare'.

Kyoto University. Dr. H. Jannasch (Woods Hole, U.S.A.) and Dr. J. Hopton (Birmingham University U.K.) have helped with advice.

We are most grateful to the Editors for fulfilling their difficult task.

The interest and financial help shown by UNESCO in this endeavour is acknowledged with much appreciation.

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October 1971

Preface

The International Biological Programme concentrated its efforts on a world-wide appraisal of the biological productivity of terrestrial and aquatic environments in relation to human welfare. Freshwater, as a separate environmental entity, has been dealt with in four preceding meetings on 'primary', 'secondary' productivity (invertebrates and fish) and the chemical environment; the corresponding publications have appeared in the form of IBP/PF Handbooks. The present treatise is concerned with microbial processes that are usually not covered in the classical limnological aspects of productivity. Methods employed in the assessment of microbial activities are, in general, remarkably different from those used in studies on plant and animal populations.

The description of methods in this book is based on contributions and discussions during the IBP/PF technical meeting on microbial production and decomposition held under the sponsorship of the Academy of Sciences of the USSR, in Leningrad on May 27–31, 1969. In this meeting, 35 aquatic microbiologists from 8 countries discussed the present status of our knowledge in microbial activities in fresh waters and methods for their quantitative assessment. In order to make the book, as far as possible, into a coherent entity, the editors found it necessary to exercise their rights. Some individual contributions are, therefore, printed with little change but others have undergone changes so as to fit them into the general pattern and still others appear only in joint chapters. The meeting was held in five sections. The title and convener of each section were:

1. Measurement of nitrogen fixation in aquatic environments (Convener, R. H. Burris).
2. Measurement of microbial decomposition of organic matter (Convener, H. Kadota).
3. Estimation of cell number and biomass of micro-organisms (Convener, V. Straškrabova).

4. Estimation of production rate of micro-organisms (Convener, Y. I. Sorokin).
5. Evaluation of the trophic role of micro-organisms (Convener, Y. I. Sorokin).

Most methods in ecology of micro-organisms in fresh waters are new or in the process of being developed. Therefore, this first attempt to collect techniques in such a new field of science obviously suffers from incompleteness due to the limited number of participants and from unevenness of scientific style. As limnology is advancing rapidly, inevitably the methods described in this book will be modified and improved.

We are indebted by Dr. Julian Rzóska, Scientific Coordinator of IBP/PF for his constant help throughout the meeting and in editing this book. We are also grateful to Professors G. G. Winberg and O. N. Bauer of the Academy of Sciences of the USSR for their kind hospitality during the meeting. We are also very grateful to Dr. H. W. Jannasch for his kind help in editing some of the manuscripts. Dr. J. W. Hopton, of the University of Birmingham, has read the script critically as to style.

We acknowledge with gratitude the considerable help for our task from UNESCO.

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1
Introduction

The role of micro-organisms in biological production in aquatic environments is complex and difficult to establish theoretically as well as methodologically.

It has been a major problem in aquatic microbiology to establish a common terminological basis for describing limnological and bacteriological processes in order to facilitate effective cooperation between hydrobiologists and microbiologists. The term 'microbial production' has been chosen for practical reasons and requires a definition.

In contrast to plants and animals, micro-organisms are not restricted to a single metabolic type but include various groups of photosynthetic, chemosynthetic, and heterotrophic organisms. Consequently, microbial production consists of primary and secondary production at the same time.

Compared to the extensive primary production by phytoplankton in fresh water, photosynthetic activity of micro-organisms is restricted to a limited area and very specific environmental conditions. Photosynthetic microbial production is of importance only in environments where light energy and appropriate electron donors such as H_2S, are simultaneously available.

Micro-organisms often enhance the productivity of water bodies by making available to organisms living there, organic matter originally produced in the surrounding areas and transported into the water body by the movement of water. Thus, in some aquatic ecosystems, besides the primary production by photosynthesis, microbial production at the expense of allochthonous organic matter cannot be neglected as a contribution to the food chain. In some water bodies, which have large surrounding drainage basins, the production of micro-organisms at the expense of energy of allochthonous materials from land or from other water bodies can be of the same order of magnitude as autochthonous primary production by plants and can sometimes exceed it.

Secondary production by heterotrophic micro-organisms will be of great quantitative importance in most situations. Compared to the secondary production by animals, microbial secondary production is of special importance for two reasons: (1) micro-organisms are capable of attacking organic substrates that cannot be utilized by animals, and (2) micro-organisms produce particulate food materials from dissolved organic materials and, therefore, represent an important link in the natural food chain.

During secondary production, decomposition processes release the energy necessary for biosynthesis, and release also mineralized nutrients for primary production. For these two important reasons, microbial production cannot be separated from microbial decomposition, neither in theoretical treatments nor from practical considerations.

From the above considerations it is quite obvious that micro-organisms are of importance in the processes of mineralization and nutrient regeneration as well as in the creation of the basic food resources in the aquatic environment. Therefore the development of the methods of evaluation of the microbial production and decomposition is now extremely important in the study of ecosystems in fresh waters.

Microbial processes of nitrification, denitrification, oxidation of inorganic sulfur compounds, and sulfate reduction are also of importance for the evaluation of biological productivity. But these transformations are not specifically treated in this text.

Besides bacteria, other micro-organisms such as moulds, yeasts, streptomyces and viruses can play an important role in some aquatic ecosystems. These organisms, however, are also not treated, since information about them from an ecological point of view is still limited.

2

Measurement of Biological N_2 Fixation with $^{15}N_2$ and Acetylene

Although the importance of biological N_2 fixation is obvious in the agricultural economy and in aquatic systems, the methods for its quantitation have been so deficient that its adequate evaluation has never been possible. The reduction of acetylene to ethylene can now serve as an index of N_2 fixation to furnish quantitative measurements of N_2 fixation in the field.

2.1 Introduction

Schöllhorn and Burris (1966) reported that azide and acetylene were reduced by the N_2-fixing enzyme complex. Independently, Dilworth (1966) also found that acetylene was reduced and demonstrated that ethylene was the product of the reduction.

An extensive application of the acetylene reduction method for field studies was reported by Stewart, Fitzgerald and Burris (1967) who employed the method to examine N_2 fixation in soil, in excised nodules from leguminous and non-leguminous plants, and in blue-green algae in lakes. In 1968 Hardy *et al.* reported that they also had used the method for investigation of N_2 fixation in soils and in leguminous nodules; they employed syringes as their vessels for exposure of samples.

The acetylene reduction method for measuring N_2 fixation in aqueous environments and in the soil by free-living and symbiotic systems is particularly attractive, because it is simple, cheap, and extremely sensitive. The opinion is generally held that a measurement of acetylene reduction to ethylene can be employed as a valid index of N_2 fixation based upon the observations that: (1) the reduction of acetylene, like the reduction of N_2, requires ATP and a reducing agent such as dithionite or reduced ferredoxin; (2) as the enzyme system is purified for N_2 fixation it is purified for C_2H_2 reduction in a parallel fashion; (3) inactivation of N_2-fixing capacity is accompanied by inactivation of C_2H_2-reducing capacity; (4) the Fe protein