HANDBOOK OF PHYCOLOGICAL METHODS

Physiological & Biochemical Methods

EDITED BY
JOHAN A. HELLEBUST
JAMES S. CRAIGIE

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PHYSIOLOGICAL AND BIOCHEMICAL METHODS

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This volume of the *Handbook of Phycological Methods* is the first compilation of biochemical and physiological procedures selected specifically for the experimental phycologist. Algae present unique problems to the researcher because of their diverse structure and composition, which differ significantly from those of other commonly used organisms. Readers interested in processes such as photosynthesis, respiration, the uptake of organic substances, transport and accumulation of ions, the use of inhibitors, isolation of enzymes, preparation of organelles and membranes, and the analysis of major constituents of algae will find practical examples presented by contributors who have applied these techniques to algae. The methods are described in sufficient detail so that they may be used by technicians, graduate and undergraduate students, and nonspecialists. Beginners and experts will appreciate the numerous technical suggestions included in the chapters.

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Editors' preface

In 1967 a special editorial committee was struck by the Phycological Society of America to consider the publication of a source book on methods in experimental phycology. The editorial committee eventually proposed the publication of four volumes to include methods from four main subdivisions of experimental phycology: culture techniques and growth measurements, cytological methods, biochemical and physiological methods, and field-oriented methods. The first volume (although not formally called Volume I) of the proposed *Handbook of Phycological Methods – Culture Methods and Growth Measurements* – was edited by Professor Janet R. Stein, University of British Columbia, and published by Cambridge University Press in 1973. It was received by experimental phycologists as a valuable source of techniques.

The editing of the present volume – Volume II, *Physiological and Biochemical Methods* – was undertaken in 1975 and has progressed smoothly, although not as rapidly as we had hoped. We gratefully acknowledge the practical and timely advice of Dr. Janet Stein. Her cooperation together with that of other members of the editorial committee for this volume lightened our editorial task considerably.

We were pleasantly surprised by the alacrity of the response of phycologists from whom we solicited contributions to this volume. Our authors made sincere efforts to meet the relatively early deadline and for this we are most appreciative. Several excellent phycologists volunteered to contribute additional chapters on interesting and useful techniques. We regret that the majority of these could not be included owing to severe space limitations.

Cambridge University Press has been most cooperative during the entire editorial process. We particularly thank Mr. Kenneth I. Werner for this help.

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Contributors *page* ix Editors' preface *page* xiii

Introduction *page* 1

I ISOLATION OF ORGANELLES AND MEMBRANES 3

1 Chloroplasts from *Euglena gracilis* 5
C. A. Price
2 Nuclei and chromatin from *Euglena gracilis* 15
D. E. Buetow
3 Mitochondria from *Chlorella* 25
Neil G. Grant
4 Microbodies from *Chlorogonium* and *Spirogyra* 31
Helmut Stabenau
5 Isolation and characterization of diatom membranes 39
Cornelius W. Sullivan

II ANALYSIS OF CHEMICAL CONSTITUENTS 57

6 Chlorophylls and carotenoids 59
Arne Jensen
7 Algal biliproteins 71
Harold W. Siegelman and J. Helen Kycia
8 Nucleic acids 81
Rose Ann Cattolico
9 Protein determination by dye binding 91
Gary Kochert
10 Carbohydrate determination by the phenol–sulfuric acid method 95
Gary Kochert

11 Glycerolipids and fatty acids of algae 99
J. P. Williams
12 Carrageenans and agars 109
J. S. Craigie and C. Leigh
13 β-1,3-Glucans in diatoms and brown seaweeds 133
Sverre Myklestad
14 Alginic acid 143
Bjørn Larsen
15 Fucoidan 151
Bjørn Larsen
16 Phenolic compounds in brown and red algae 157
Mark A. Ragan and J. S. Craigie
17 Brown seaweeds: analysis of ash, fiber, iodine, and mannitol 181
Bjørn Larsen
18 Quantitation of the macromolecular components of microalgae 189
Gary Kochert
19 ATP, ADP, and AMP determinations in water samples and algal cultures 197
David M. Karl and Osmund Holm-Hansen

III ENZYMES 207

20 Ribulose-bis-phosphate carboxylase in *Chlamydomonas* 209
S. S. Badour
21 Nitrate reductase in marine phytoplankton 217
Richard W. Eppley
22 Threonine deaminase in marine microalgae 225
N. J. Antia and R. S. Kripps
23 Phosphoenolpyruvate carboxylase of blue-green algae 233
Brian Colman
24 Aldolases of multicellular marine algae 239
Kazutosi Nisizawa
25 The glucosyltransferases of red algae 245
Jerome F. Fredrick

26 Anion-activated adenosine triphosphatases 255
Paul G. Falkowski
27 Extracellular acid phosphatase of *Ochromonas danica* 263
N. J. Patni and S. Aaronson

IV PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES 267

28 Determination of photosynthetic rates and ^{14}C photoassimilatory products of brown seaweeds 269
Bruno P. Kremer
29 Polarographic measurements of photosynthesis and respiration 285
Alan D. Jassby
30 Polarographic measurements of respiration following light-dark transitions 297
Alan D. Jassby
31 Hill reaction and photophosphorylation with chloroplast preparations from *Chlamydomonas reinhardi* 305
Stephen Lien
32 Respiratory assimilation of ^{14}C-labeled substrates by a microalga 317
Keith E. Cooksey
33 Respiratory processes in mitochondria 329
Neil G. Grant
34 Determination of protein synthesis during synchronous growth 337
Dennis J. O'Kane and Raymond F. Jones
35 Nucleic acid synthesis 353
Rose Ann Cattolico
36 Nitrogen fixation 363
Timothy H. Mague
37 Uptake of organic substrates 379
Johan A. Hellebust and Yi-Hung Lin
38 Release of organic substances 389
C. Nalewajko

V NUTRIENTS 399

39 Nitrate uptake 401
Richard W. Eppley
40 Phosphate uptake 411
F. P. Healey
41 Sulfate uptake 419
Monica Lik-Shing Tsang, Robert C. Hodson, and Jerome A. Schiff
42 Uptake of silicic acid 427
Farooq Azam and Sallie W. Chisholm
43 Uptake of micronutrients and toxic metals 435
Anthony G. Davies

VI ION CONTENT AND TRANSPORT 447

44 Measurement of ionic content and fluxes in microalgae 449
J. Barber
45 Ion transport and ion-stimulated adenosine triphosphatases 463
Cornelius W. Sullivan

VII INHIBITORS 477

46 Inhibitors used in studies of algal metabolism 479
S. S. Badour

VIII APPENDIX 489

List of suppliers 491

IX INDEXES

Subject 495
Author 500
Taxonomic 511

Introduction

We have attempted to present as many useful methods as possible for physiological and biochemical investigations with algae. In view of the extreme diversity of the algae, ranging from blue-green bacteria-like organisms to large complex seaweeds, it is clearly impossible to include in a single volume methods applicable to all situations. We have therefore selected methods that cover a broad range of experimental procedures and as wide a variety of algal types as possible. Most of these are described in sufficient detail to be useful to scientists with relatively little previous experience in experimental phycology. We realize that methods which apply to all types of algae or to algae in different physiological conditions are not yet available. In such cases the authors have described the limitations of their techniques and have included brief discussions of how procedures may be modified to suit different algae or other conditions. References to more advanced or specialized techniques, or to methods for other algal species, are frequently included in the various chapters. The brief list of references at the end of this introduction will provide the advanced student with information and special techniques, many of which may be applied in phycological research.

Names of the suppliers of most of the specialized materials and equipment necessary for the various methods are cited, and the reader should consult the appendix for addresses. Sources of equipment and materials are published annually in the United States in *Science* (American Association for the Advancement of Science, 1515 Massachusetts Ave., N.W., Washington, D.C. 20005) and in Canada in *Research and Development* (Maclean Hunter, 418 University Ave., Toronto 101, Ontario M5W 1A7) and *Laboratory Products News* (Southam Business Publications Ltd., 1450 Don Mills Rd., Don Mills, Ontario M3B 2X7).

In most cases the sources of the algae described in this handbook are given. The clone number of the species is provided when known, followed by the acronym for the algal culture collection from which the clone was obtained (e.g., UTEX, see Appendix: List of Suppliers).

We hope this handbook will find a wide variety of users. It should serve as a useful source of techniques for graduate students and scien-

tists who may occasionally use algae as experimental material, as well as for specialists in experimental phycology. Many of the procedures described in this volume are suitable for courses in experimental phycology. Several chapters have been included for those interested in the industrially important chemical components of seaweeds. Although field techniques will be treated in a subsequent handbook, the present volume includes several chapters of interest to scientists concerned with field investigations as well as with the effects of toxic metals on algae.

General references

Bergmeyer, H. U. 1974. *Methods of Enzymatic Analysis,* 2nd English ed., vols. 1–4. Academic Press, New York.

Colowick, S. P., and Kaplan, N. O. (eds.). 1955–75. *Methods in Enzymology,* vols. 1–43. Academic Press, New York. (Especially vols. 23 and 24 on photosynthesis and nitrogen fixation are of interest as they deal extensively with algal material.)

Dawson, R. M. C., Elliot, D. C., Elliot, W. H., and Jones, K. M. (eds.). 1969. *Data for Biochemical Research,* 2nd ed. Oxford University Press, London. 654 pp.

Herbert, D., Phipps, P. J., and Strange, R. E. 1971. Chemical analysis of microbial cells. *Methods Microbiol.* 5B, 209–344.

Lowry, O. H., and Passonneau, J. V. 1972. *A Flexible System of Enzymatic Analysis.* Academic Press, New York. 291 pp.

Prescott, D. M. (ed.). 1975. Yeast cells. *Methods Cell Biol.* 12, 1–395.

Umbreit, W. W., Burris, R. H., and Stauffer, J. F. 1972. *Manometric and Biochemical Techniques,* 5th ed. Burgess, Minneapolis. 387 pp.

Whistler, R. L., and BeMiller, J. N. (eds.). 1964–76. *Methods in Carbohydrate Chemistry,* vols. 4–7. Academic Press, New York.

Whistler, R. L., and Wolfrom, M. L. (eds.). 1962–3. *Methods in Carbohydrate Chemistry,* vols. 1–3. Academic Press, New York.

Section I

Isolation of organelles and membranes