# BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY HANDBOOK

Bernard Atkinson
Ferda Mavituna

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The Nature Press

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## DEDICATION

This Handbook is dedicated to all those unknown and unnamed people whose efforts over the years have created Biochemical Engineering and Biotechnology, and who have opened up the possibilities which lie before us.



Published in the United Kingdom by MACMILLAN PUBLISHERS LTD
(Journals Division), 1983
Distributed by Globe Book Services Ltd
Canada Road, Byfleet, Surrey, KT14 7JL, England

ISBN 0 333 33274 1

Published in the USA and Canada by THE NATURE PRESS, 1983.
15 East 26th Street, New York, N.Y. 10010

**Library of Congress Cataloging in Publication Data**
Atkinson, Bernard, 1936-
Biochemical engineering and biotechnology handbook.
Includes index.
1. Biochemical engineering. 2. Bioengineering.
I. Mavituna, Ferda, 1951– II. Title.
TP248.3.A853 1983 660'.6 82-14462
ISBN 0-943818-02-8

Printed in Great Britain by
St Edmundsbury Press, Bury St Edmunds, Suffolk.

Filmset by Filmtype Services Limited, Scarborough, North Yorkshire.

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# Preface

The current interest in biotechnology and the belief that it will expand very considerably is based upon three factors:

(i) The raw materials can be obtained from renewable resources.

(ii) Biotechnological processes appear likely to be economical against the chemical processing of vegetable materials.

(iii) A wide range of possibly valuable products is being defined by both traditional biological methods and through genetic manipulation.

As with most areas of technology, local and global political considerations distort both the areas of development and the rates of progress, e.g. alcohol fermentations are important independent of the overall economics. In general biotechnology can be divided into three areas defined as large, medium and small; terms which refer to the scale of industrial development rather than the size of the individual production units which might be built:

(i) Large scale, where biotechnology must compete with petroleum and coal, as a primary source of carbon compounds for fuels and high tonnage industrial products.

(ii) Medium scale, where biotechnology must compete with both petroleum-style technology (whether the carbon source is petroleum, coal or vegetable) to produce either the commodity chemicals currently in use or substitutes for them, and with agriculture to produce natural products such as proteins and lipids.

(iii) Small scale where specifically biochemical products are produced for which no other routes can currently be foreseen.

Small scale processes seem certain to continue their present rapid proliferation and growth; their products offer such improvements in medical practice and for industrial processes, that the cost of the products is not a deciding factor in whether the products are developed but in which manufacturers succeed. The medium and large scale processes are probably not currently viable economically except in a few cases, e.g. some organic acids where production is truly economic, alcoholic drinks, and acetic acid for human consumption. However, it is likely that the next twenty years will see the establishment of large scale microbiological processes with vegetable materials as feedstock (Fig. 1). These processes will:

(i) Produce primary materials as fuels and feedstocks for conversion to commodity chemicals, though whether the later conversion is chemical or microbiological will depend upon processing economics.

(ii) Produce a range of commodity chemicals directly from vegetable substances rather than via some primary product such as ethanol.

At the large and medium scale the 'market' value of many potential biological products is well-established, since the objective is either direct substitution or provision of material with properties similar to an available product. The cost of raw materials is also likely to be known. The difference between cost and value defines the allowable process and marketing expenditure, and serves to target research and development, whether biological or process engineering in emphasis, to meet these needs.

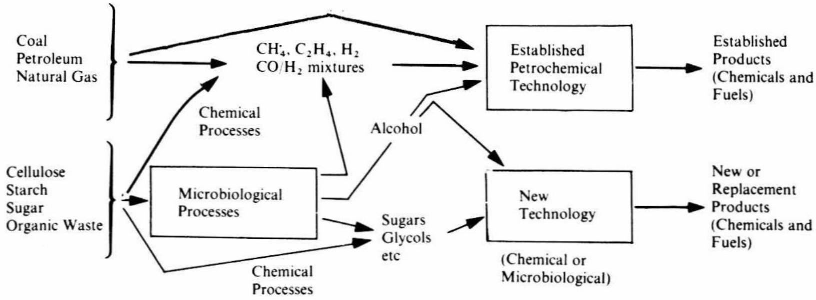


Figure 1. The competitive routes to large tonnage products.

The *Biochemical Engineering and Biotechnology Handbook* is intended to assist the development of both large and small biotechnological processes by establishing for the unit processes and unit operations involved:

1) their scientific and engineering basis;

2) their performance and operating characteristics;

3) the factors which influence their performance;

4) their integration into complete processes.

Such information allows scientists and engineers:

1) to ensure the appropriateness of research and development in meeting overall process objectives;

2) to estimate the extent to which particular processes are sub-economic and the possibilities for research and development to change this situation;

3) to establish the appropriate methodology for particular process development;

4) to identify equipment needs.

The Handbook has been structured into fourteen chapters detailed contents of each chapter are given on p.v, and a brief glossary appears at the beginning of the chapter (as appropriate). Extensive sub-headings have been provided and these have been used to form the basis of an index (p.1115). Within each chapter information has been presented predominantly as tables and figures so that those knowledgeable in a discipline appropriate to biotechnology can readily identify relevant subject matter. The emphasis is placed on the 'what' and 'how' of biological processes, for those who require detailed explanation of the 'why' literature references and bibliography sources have been provided.

Biotechnology will become an important, as opposed to peripheral, part of the industrial scene if it meets the needs of the community at a price the community can afford. Frenzy, fashion and invocation of crisis, whether of food or energy, are not sustainable. Rather facts, hard work, commitment, confidence, professionalism and the pleasure of invention are the substance by which biotechnological processes can make a modest, though significant contribution to material well-being.

B. Atkinson
F. Mavituna
August 1982.

# Acknowledgements

In 1976 Mr. A.N. Emery of the Department of Chemical Engineering, University of Birmingham, produced *Biochemical Engineering – a Report Prepared for the Science Research Council and the Institution of Chemical Engineers*. One of the recommendations contained in the report highlighted the need for a handbook of biochemical engineering data. Subsequently the Science and Engineering Research Council, formerly SRC, with the encouragement of the Research Committee of I.Chem. E., provided funding which allowed one of the authors (F.M.) to lay the groundwork for the *Biochemical Engineering and Biotechnology Handbook*.

The authors would like to express their appreciation for the encouragement and direct assistance given by members of the various SERC/I.Chem.E. Committees, and in particular to the Science and Engineering Research Council and Mr. Emery.

The awareness of the authors as to the importance of downstream processing within biochemical engineering was greatly enhanced by the work of one of them (B.A.) in conjunction with Mr. Philip Sainter, on a project entitled 'Technological Forecasting for Downstream Processing in Biotechnology' which formed part of the EEC Forecasting and Assessment in the Field of Science and Technology (Biosociety Sub-Programme). The authors would particularly like to thank Mr. Sainter for his help in conjunction with Chapters 12 and 13.

The form of the Handbook developed over an extensive period but was helped particularly by Dr. Ann Ralph, whose knowledge of biochemistry and previous work on chemical engineering texts was invaluable. The Handbook owes much to Dr. Ralph's patience and attention to detail and to the way in which she undertook the difficult editorial task of converting the manuscript into a harmonious whole.

Appreciation is also due to Rosemary Foster of Macmillan whose charming perseverance contributed greatly to maintaining the momentum of the project.

Thanks are also due to Mrs. Nora Taylor, the Department of Chemical Engineering Librarian at UMIST who was a mine of information on the complexities of library and retrieval systems and with the checking of occasional abstruse references.

Finally, the authors would like to express their gratitude to individuals and organisations too numerous to detail, who by discussion and direct assistance helped them considerably in clarifying their ideas and hopefully achieving a rounded view of the problems inherent in the industrial application of biochemical engineering and biotechnology.

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# CHAPTER 1

# PROPERTIES OF INDUSTRIALLY IMPORTANT MICROORGANISMS

## GLOSSARY

**Activated sludge** Material containing a very large, active microbial population, used in the purification of waste water.
**Adaptation** Ability to exist in a changed environment.
**Aerobe** Microorganism that requires molecular oxygen.
**Algae** Group of simple, mainly aquatic plants capable of **photosynthesis**.
**Anaerobe** Microorganism that grows in the absence of molecular oxygen.
**Bacillus** Rod-shaped bacterium.
**Budding** Form of asexual reproduction.
**Capsule** Gelatinous layer surrounding the cell wall of many bacteria.
**Chlorophyll** Green pigment of plants consisting of closely related colouring components, chlorophyll *a* and chlorophyll *b*, etc.
**Chloroplast** Body in a plant cell containing **chlorophyll**.
**Chromosome** Thread-shaped structure bearing genes located in the nucleus.
**Coccus** Spherical bacterium.
**Culture** Population of **microorganisms**.
**Cytoplasm** Fluid contained within the cell membrane excluding the **nucleus**.
**Diploid** Used to describe cells having **chromosomes** in pairs.
**Ecology** Study of an **organism** in relation to its environment.
**Enzyme** Protein-based catalyst produced within an organism.
**Facultative anaerobe** Bacterium that grows under either anaerobic or aerobic conditions.
**Fermentation** Commonly: any industrial microbiological process. Specifically: anaerobic microbiological processes.
**Fermenter** Industrial microbiological reactor.
**Gram's stain** Used for the differentiation of bacteria.
**Growth curve** Graphical representation of the growth of an **organism** in nutrient medium in a batch reactor.
**Haploid** Used to describe cells having a single set of **chromosomes**.
**Heterotroph** Microorganism that uses organic compounds as a carbon source.
**Medium** Mixture of **nutrient** substances.
**Meiosis** Form of cell division in which a **diploid** cell divides giving rise to two **haploid** cells.
**Metabolism** Overall process by which an **organism** uses **nutrients**.
**Metabolite** Product of biochemical activity.
**Microaerophile** Bacterium that grows most rapidly in the presence of small amounts of molecular oxygen.
**Microbial film** Adherent aggregate of **microorganisms** attached to a supporting surface.
**Microbial floc** Adherent aggregate of **microorganisms** in suspension.
**Microorganism** Form of life of microscopic dimensions.
**Mitochondria** Minute granular, rod-like structures contained within the **cytoplasm**.
**Mitosis** Process by which cell divides into two daughter cells each having an identical complement of genetic information.
**Mixed culture** Two or more species of **microorganisms** living in the same medium.
**Morphology** Structure and forms of an

organism.
**Mutation** Stable change of gene inherited on reproduction.
**Nucleus** Dense inner mass of the cell enclosed within a membrane.
**Nutrient** Substance used as food.
**Obligate** Necessary or required.
**Organism** Living biological specimen.
**Parasite** Living **organism** deriving its nutrition from another living organism.
**Photosynthesis** Production of glucose from carbon dioxide and water in the presence of **chlorophyll** with absorption of light.
**Physiology** Study of the function of **organisms**.
**Plasmid** Hereditary unit contained in the **cytoplasm**.
**Protein** Class of organic compounds associated with living matter, based on a combination of amino acids.
**Protozoa** Unicellular animals.
**Pure culture** Culture containing only one species of **microorganism**.
**Respiration** Any chemical reaction whereby energy is released for use by the **organism**.
**Sacrophyte** Organism that utilizes organic matter in solution from dead or decaying plant or animal tissue.
**Slime layer** Gelatinous covering of the cell wall, used synchronously with capsule.
**Species** One kind of **organism**.
**Spore** Minute, thick-walled, resistant body that forms within the cell and is considered as the resting stage.
**Sterile** Free of **viable microorganisms**.
**Strain** Pure culture of **microorganisms** composed of the descendants of a single **organism**.
**Substrate** Substance acted upon by an **enzyme**.
**Taxonomy** Classification of **organisms**.
**Tissue** Collection of cells forming a structure.
**Vacuole** Droplet in a cell often containing reserve food material.
**Viable** Capable of growth.
**Virus** Parasitic microorganism smaller than a bacterium.
**Zoogloeal masses** Microbial film or microbial floc, normally associated with biological waste water treatment.

# GENERAL CLASSIFICATION OF MICROORGANISMS

A classified arrangement of microorganisms according to their mutual affinities or similarities permits a logical and informative system of naming. It may also be used as a key for the identification of organisms. Depending on the criteria used for grouping, a particular classification may show the evolutionary relationship between organisms (i.e. natural classification) or may be made for a single, defined purpose, e.g., to assist in finding the answers to specific questions (i.e special or artificial classification).

For each of the main groups of microorganisms, the taxonomic hierarchy is a system in which a single, all-inclusive category is divided and subdivided into progressively smaller and less-inclusive categories. Scheme 1 shows the most important categories of the taxonomic hierarchy arranged in the order in which they must be employed.

SCHEME 1

Kingdom→Division (or Phylum)→Class→Order→Family→Genus→Species→Strain

The kingdom Protista (*see* Fig. 1) comprises unicellular organisms capable of self-duplication or of directing their own replication. Procaryotes do not possess a true nucleus or a nuclear membrane, while eucaryotes do have a true nucleus enclosed within a distinct nuclear membrane. Some features distinguishing between procaryotic and eucaryotic cells are summarized in Table 1. The noncellular protists (i.e. viruses) do not undergo self-replication, instead they direct their reproduction within another cell termed the host.

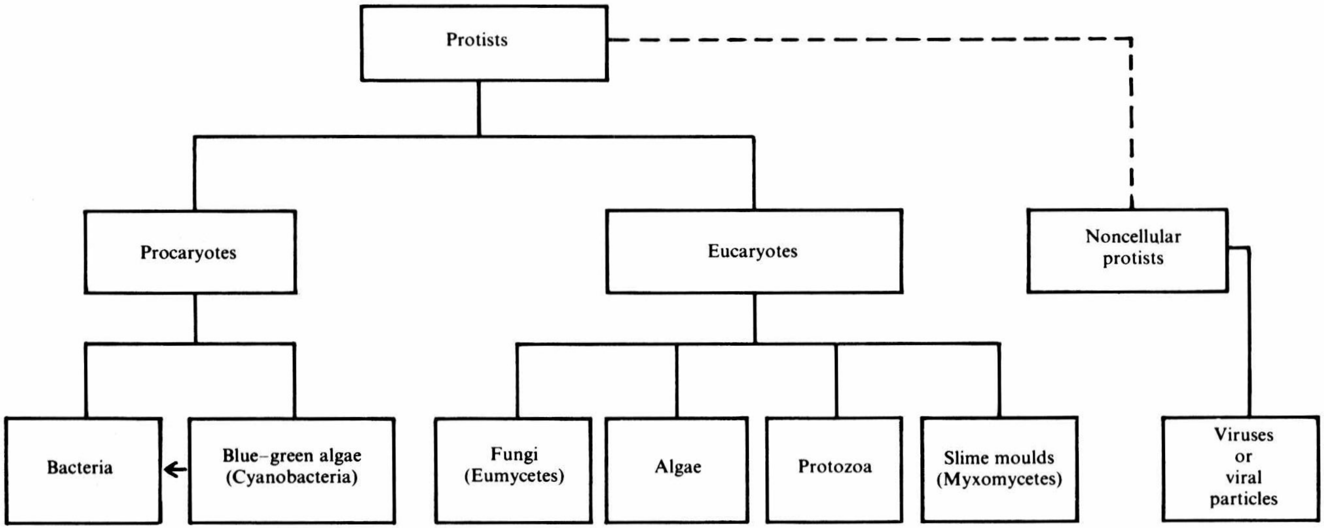


Figure 1. The kingdom of protists. Blue–green algae in this scheme have been classified as a separate group of microorganisms, although they are frequently considered to be cyanobacteria and included with other bacteria. Alternatively blue–green algae are classified as cyanophycophyta (*see* Table 6.).

Table 1. Features distinguishing procaryotic and eucaryotic cells.

| Feature | Procaryotic cells | Eucaryotic cells |
|---|---|---|
| Size of organism | $< 1$–$2 \times 1$–$4$ μm | $> 5$ μm in width or diam |
| Genetic system | | |
| Location | Nucleoid, chromatin body or nuclear material | Nucleus, mitochondria, chloroplasts |
| Structure of nucleus | Not bounded by nuclear membrane. One circular chromosome | Bounded by nuclear membrane. One, or more, linear chromosome |
| | Chromosome does not contain histones. No mitotic division | Chromosomes have histones. Mitotic nuclear division |
| | Nucleolus absent. Functionally related genes may be clustered | Nucleolus present. Functionally related genes not clustered |
| Sexuality | Zygote is partially diploid (merozygotic) | Zygote is diploid |
| Cytoplasmic nature and structures | | |
| Cytoplasmic streaming | Absent | Present |
| Pinocytosis | Absent | Present |
| Gas vacuoles | Can be present | Absent |
| Mesosome | Present | Absent |
| Ribosomes | 70S,[a] distributed in the cytoplasm | 80S arrayed on membranes (e.g., endoplasmic reticulum). 70S in mitochondria and chloroplasts |
| Mitochondria | Absent | Present |
| Chloroplasts | Absent | May be present |
| Golgi structures | Absent | Present |
| Endoplasmic reticulum | Absent | Present |
| Membrane-bound (true) vacuoles | Absent | Present |
| Outer cell structures | | |
| Cytoplasmic membranes | Generally do not contain sterols. Contain part of respiratory and, in some, photosynthetic machinery | Sterols present. Do not carry out respiration and photosynthesis |
| Cell wall | Peptidoglycan (murein or mucopeptide) as component | Absence of peptidoglycan |
| Locomotor organelles | Simple fibril | Multifibrilled with microtubules |
| Pseudopodia | Absent | May be present |
| Metabolic mechanisms | Varied, particularly that of anaerobic energy-yielding reactions. Some fix atmospheric $N_2$. Some accumulate poly-β-hydroxybutyrate as reserve material | Glycolysis is pathway for anaerobic energy-yielding mechanism |
| DNA base ratios (G + C%) | 28–73 | About 40 |

[a] S refers to the Svedberg unit, the sedimentation coefficient of a particle in the ultracentrifuge.

Table 2. Some criteria used for classifying microorganisms.

| Microorganisms | Criteria |
|---|---|
| Bacteria | Morphology, staining reactions, spore formation, motility, antigenic structure, metabolism |
| Fungi | Nature of the thallus, form of sexual/asexual reproductive structures |
| Aigae | Types of pigment, number and arrangement of flagella on motile cells, nature of storage carbohydrates |
| Protozoa | Presence of flagella or cilia, modes of locomotion and/or reproduction, presence and nature of specialized intracellular structures or skeletal structures |
| Viruses | Type of genome (DNA or RNA), size of the virion, presence of envelope |

## Detailed Classification of Bacteria

Type 1 Phototrophic bacteria

- Order I Rhodospirillales
  - Suborder Rhodospirillineae
    - Family I Rhodospirillaceae
      - Genus I *Rhodospirillum*
      - Genus II *Rhodopseudomonas*
      - Genus III *Rhodomicrobium*
    - Family II Chromatiaceae
      - Genus I *Chromatium*
      - Genus II *Thiocystis*
      - Genus III *Thiosarcina*
      - Genus IV *Thiospirillum*
      - Genus V *Thiocapsa*
      - Genus VI *Lamprocystis*
      - Genus VII *Thiodictyon*
      - Genus VIII *Thiopedia*
      - Genus IX *Amoebobacter*
      - Genus X *Ectothiorhodospira*
  - Suborder Chlorobiineae
    - Family III Chlorobiaceae
      - Genus I *Chlorobium*
      - Genus II *Prosthecochloris*
      - Genus III *Chloropseudomonas*
      - Genus IV *Pelodictyon*
      - Genus V *Clathrochloris*
  - Addenda
      - Genus *Chlorochromatium*
      - Genus *Cylindrogloea*
      - Genus *Chlorobacterium*

Type 2 Gliding bacteria

- Order I Myxobacterales
  - Family I Myxococcaceae
    - Genus I *Myxococcus*
  - Family II Archangiaceae
    - Genus I *Archangium*
  - Family III Cystobacteraceae
    - Genus I *Cystobacter*
    - Genus II *Melittangium*
    - Genus III *Stigmatella*
  - Family IV Polyangiaceae
    - Genus I *Polyangium*
    - Genus II *Nannocystis*
    - Genus III *Chondromyces*
- Order II Cytophagales
  - Family I Cytophagaceae
    - Genus I *Cytophaga*
    - Genus II *Flexibacter*
    - Genus III *Herpetosiphon*
    - Genus IV *Flexithrix*
    - Genus V *Saprospira*
    - Genus VI *Sporocytophaga*
  - Family II Beggiatoaceae
    - Genus I *Beggiatoa*
    - Genus II *Vitreoscilla*
    - Genus III *Thioploca*
  - Family III Simonsiellaceae
    - Genus I *Simonsiella*
    - Genus II *Alysiella*
  - Family IV Leucotrichaceae
    - Genus I *Leucothrix*
    - Genus II *Thiothrix*
  - Incertae sedis
    - Genus *Toxothrix*
  - Familiae incertae sedis
  - Achromatiaceae
    - Genus *Achromatium*
  - Pelonemataceae
    - Genus I *Pelonema*
    - Genus II *Achroonema*
    - Genus III *Peloploca*
    - Genus IV *Desmanthos*

Type 3 Sheathed bacteria

- Genus *Sphaerotilus*
- Genus *Leptothrix*
- Genus *Streptothrix*
- Genus *Lieskeela*
- Genus *Phragmidiothrix*
- Genus *Crenothrix*
- Genus *Clonothrix*