## Biotechnology

A Comprehensive Treatise in 8 Volumes edited by H.-J. Rehm and G. Reed

Volume 3
Volume Editor: H. Deliweg

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## Volume 3

Biomass, Microorganisms for Special Applications, Microbial Products I, Energy from Renewable Resources

Volume Editor: H. Dellweg



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## Preface to the Series

The title of this series of books "Biotechnology - A Comprehensive Treatise in Eight Volumes" is truly descriptive of its contents. Biotechnology is very much an interdisciplinary field linking at least three different branches of science: Microbiology, biochemistry, and engineering. Within the past twenty years there has been generous assistance from genetics and molecular biology. Therefore, an understanding between experts in the various disciplines of the field is essential to the successful application of its basic concepts. It is the ambitious aim of this series to promote this understanding.

"Biotechnology" hopes to be comprehensive in two different senses. First, it spans the field from its basic concepts to practical industrial applications. Secondly, it spans a gamut of widely differing applications, from microbial transformations to the production of enzymes, biomass, primary and secondary products of microbial metabolism, to the production of foods and feeds, and to sewage disposal and other microbial degradations.

"Biotechnology" may serve as a reference work, as a comprehensive description of the state of the art, as well as a guide to the original literature. It is specifically directed to microbiologists, biochemists, bioengineers, chemical engineers, and food and pharmaceutical chemists working in industry, at universities or at public institutions.

Biotechnology" consists of eight volumes. The first two volumes form the basic concepts of microbial sciences and bioengineering. The third and fourth volume describe the industrially important products of microbial metabolism as well as the production of biomass. The fifth volume deals with the production of foods and feeds with the aid of microbes. The sixth volume will be divided into Parts 6a and 6b to cover microbial transformations and special microbial processes, respectively. The seventh volume is devoted to the production and uses of free enzymes. The final volume

deals with biodegradations.

The authors of individual chapters have been chosen because of their recognized expertise and their contributions to the field of biotechnology. Their willingness to impart this knowledge to their colleagues forms the basis of "Biotechnology", and this is gratefully acknowledged by the editors. This work could not have been brought to fruition without the foresight and the constant and diligent support of the publisher. We are grateful to Verlag Chemie for publishing "Biotechnology" with the excellence which readers have become accustomed to expect from this publisher. Special thanks are due Dr. Hans F. Ebel. the scientific editor of Verlag Chemie, who conceived the idea of "Biotechnology" and without whose constant efforts the series could not be published. Finally, the editors wish to thank the members of the Editorial Advisory Board for their encouragement, their helpful suggestions and their criticism.

With this short introduction the editors entrust "Biotechnology" to their colleagues; for their use, their enjoyment, and their critical consideration.

H.-J. Rehm

G. Reed

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Biotechnology is an interdisciplinary field. It is largely oriented towards the industrial application of single-cell organisms (or parts thereof) for the creation of products or the conversion of materials. This volume of "Biotechnology" deals with product formation.

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The most direct "product" is the microorganism itself through multiplication. The production of "single-cell biomass" is described in Chapters 1a through 1e. Processes of this kind sometimes have a long tradition, such as the production of baker's yeast. The classical substrates are sugars, especially sucrose in cane or beet molasses. With decreasing availability of molasses other carbohydrate sources may become economically interesting, such as whey or by-products from corn starch saccharification.

In the case of baker's yeast capabilities of the microorganism, such as dough raising capacity and keeping quality, are most desired and therefore considerations regarding substrate, production process and even the kind of microorganism rank second. On the other hand, the production of biomass for feeding purposes is mainly geared to using the cheapest available substrate. In most cases it is important that the dried microorganisms have a high protein content, are low in nucleic acids, and free of hazardous components. The term "single-cell protein" (SCP) is common but inadequate, since SCP at best contains 70% protein. In its production a distinct substrate is normally used and it is often advantageous to change the microorganism when the sub-

strate has to be changed. For example, the production of fodder yeast on spent sulfite liquor - the residue from cellulose and paper manufacture - was based on the fast growing Candida utilis, which attacks more carbon and nitrogen compounds than other common yeasts (Chapter 1a). During the late sixties, higher n-alkanes (the by-products from gas oil refining) appeared to be excellent substrates for the production of food protein. In theses processes yeasts were still favored over bacteria partly for psychological reasons and partly because Candida lipolytica and C.tropicalis had been extensively studied in the laboratory and in production plants as sources of microbial protein (Chapter 1b). The situation changed with the first oil crisis in 1973, when the costs of higher alkanes were in danger of skyrocketing. Methane, the simplest alkane and readily available as natural gas was then considered as a substrate, but its low solubility in water causes technological problems. Moreover, its oxidative conversion to methanol in methylotrophic organisms is inefficient and energy consuming. Consequently methanol itself was considered as a carbon-containing substrate (Chapter 1c). It is available from several low-cost synthetic routes. Methylotrophic capabilities are frequently found with bacteria but rarely with yeasts. Accordingly, bacteria are the organisms of choice when methanol is the substrate.

There is a mession that SCP may help to

our ability to use the energy from sunlight

Biotechnology has benefited greatly from SCP processes, especially from the development of new reactor designs and recovery processes. However, SCP production was not successful even though the problems of producing high quality protein foods, free of harmful by-products, have mostly been solved. The reasons are mainly economical, political and psychological. There is no question that SCP may help to satisfy the world protein demand at a later time.

The cheapest carbon source available is carbon dioxide. Its exploitation depends on our ability to use the energy from sunlight for the conversion of carbon dioxide to useful products. For countries in the tropical and subtropical zones the solution of this problem is of vital importance (cf. Chapter 1d).

The list of processes leading to proteinrich microbial biomass would be incomplete without mentioning the cultivation of edible fungi with fruiting bodies on trunks, woodchips, grain straw and other substrates. Aspects of edible mushroom production are discussed in detail in Chapter 1e.

Microbial biomass is not only a source of food protein. In several cases microorganisms are produced for distinct applications according to their particular potentials (Chapters 2a through 2e). Baker's yeast is an example. Special yeast strains are available for the processing of alcoholic beverages, and other microorganisms are in use as starter cultures for milk and meat processing (Chapter 2a), and for other purposes (Chapter 2b). Microorganisms are, furthermore, utilized in soil amelioration: they improve the uptake of ions or substrates from the soil during plant growth, or they support the decay of materials during humus formation (Chapter 2c). Recent developments aim at replacing chemical pest control with all its undesirable side effects by microbial methods. Several bacteria and fungi are known to produce endotoxins and other compounds which are pathogenic for damaging insects. Cell or spore suspensions of such microbes are already used in agriculture (Chapter 2e).

The number of substances which may be excreted by microorganisms is almost endless. They may be classified as "simple" compounds (lower alcohols, acids) and "complicated" compounds (natural prod-

ucts) or as "primary products" and compounds evolving from "secondary metabolism". They may be distinguished according to their production volume (citric acid: 250 000 t/a, tyrosin: 100 t/a) or according to their use as solutes and "basic chemicals" and as "specialities", mainly of pharmaceutical and medical importance. All of these classifications are imcomplete and not convincing. Accordingly, difficulties arise when in a "Comprehensive Treatise of Biotechnology" microbial products are to be partitioned between two volumes. The first suggestion was to distinguish primary metabolites (Volume 3) from secondary metabolites (Volume 4). However, there are no clear definitions, and even the fermentation kinetics of some simple amino acids are not at all typical for the fermentation of primary products.

Clearly, ethanol is a simple, primary bulkware product. Thus, ethanol fermentation is described in Chapter 3a. Considering its increasing production and its importance as a basic chemical and as an engine fuel, special emphasis was placed on classical and modern raw materials as well as on recent efforts to find energy saving methods for the recovery of ethanol from the fermented mash.

There are other alcohols, carbonyl compounds and polyols that originate from fermentation processes, partly fitting the scope of Volume 3, partly that of Volume 4. It has been decided that they will all be treated in Volume 4.

Apart from ethanol, simple organic acids are covered in Chapter 3. The most important ones are citric acid (Chapter 3d) and gluconic acid (Chapter 3e). Acetic acid (Chapter 3b) and lactic acid (Chapter 3c) are also treated here with regard to the microbial, biochemical and technological aspects of their production. Both acids will be discussed again in Volume 5 in the context of food production. All other lower acids are then summarized as "organic acids of minor importance" in Chapter 3f. All amino acids are covered in Chapter 3g which was written by our Japanese colleagues as they are doubtlessly most experienced in this branch of fermentation.

Microbial polysaccharides are no "small compounds", but they are homo- or heteropolymers, composed of monomeric sugars (Chapter 3h). A chapter (3i) on microbial emulsifiers and de-emulsifiers is included. This is a very young field of biotechnological research, but nevertheless of high importance, should such compounds eventually contribute to enhanced oil recovery.

Several fermentations may lead to the large-scale production of chemicals and

high-energy fuels from renewable resources. It is the responsibility of our generation to evaluate and develop such technologies. A comparative discussion of the economy and the availability of raw materials as fermentation substrates (Chapter 4) was therefore considered appropriate in this volume of "Biotechnology".

Berlin, in November 1982 H. Dellweg

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