Interbiotech '87
Enzyme Technologies

Edited by

A. Blažej
J. Zemek

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Progress in Biotechnology
VOLUME 4

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Enzyme Technologies

Proceedings of the International Symposium on Biotechnology,
Bratislava, Czechoslovakia, June 25–26, 1987

Edited by
A. Blažej and J. Zemek
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ELSEVIER
Amsterdam – Oxford – New York – Tokyo 1988

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Elsevier Science Publishing company, Inc.
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New York, NY 10017

for the East European Socialist Countries, Democratic Republic of Vietnam, Mongolian People's Republic, People's Republic of Korea, People's Republic of China, Republic of Cuba
Alfa, Hurbanovo nám. 3, 815 89 Bratislava, Czechoslovakia

for all remaining areas
Elsevier Science Publishers
Sara Burgerhartstraat 25
P. O. Box 211, 1000 AE Amsterdam, The Netherlands

ISBN 0-444-98924-2 (Vol. 4)
ISBN 0-444-42435-0 (Series)

Printed in Czechoslovakia

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PREFACE

Enzymes and enzyme preparates have passed through a fascinating period of their development in the last twenty years in both enzyme theory and in the field of their preparation, isolation, modification and application. A new scientific and technological discipline has been established - enzyme engineering. The application of biocatalysts in industry has become a synonym for biologization of industry, characterized by lower energetic requirements, at the same time respecting the ecological impact and enabling production of industrial biochemicals with new qualities. Since the industrial application of enzymes has been mostly in the food and microbial process industry it is not surprising that the majority of preparations considered for commercial development stem from this area. Involvement of the process-engineers in biocatalytic processes have resulted in more exact mathematical formulations describing these processes. Recombinant DNA techniques - developed primarily by molecular geneticists to probe the structure, organization and function of genetic material - have increasingly been adopted by chemists and enzymologists to investigate questions about the structure and functions of enzymes. The biotechnology industry is using such techniques for the production of enzymes of new generations changed in specific amino acids in the native protein in the process of in site-directed mutagenesis. These enzymes with modified properties are of practical use in the processes where their native counterparts could not be introduced. This refers to the application of biocatalysts above all in the chemical industry. Recent results obtained with the application of enzymes in organic media, biphasic media and reversed micelles opened the way for large scale application of biocatalysts in the processes originally carried out with the classical chemical catalysts.

In the field of analytical methods, the application of enzymes enabled the introduction of highly precise, sensitive and automatic methods to replace the obsolete chemical ones. The application of modified enzymes enabled new trends in the diagnosis and therapy of internal diseases.

This volume deals with enzyme technologies and presents selected papers of the international symposium INTERBIOTECH '87 which as a concomitant scientific event accompanied the International Chemical Fair, Incheba in Bratislava. This symposium covered various fields of enzyme technologies, including the selection techniques of producing strains, preservation of the microbial producers in the collections, modification of enzymes using techniques of protein engineering, application of biospecific methods in the purification steps and application of immobilized biocatalysts in industry. In this book experience and knowledge of enzyme technology are represented by some typical contributions.

We are indebted to all authors for their preparation of these manuscipts and their cooperation in the Symposium. We apologize for any errors or omissions in the text that escaped our scrutiny. Appreciation is due to representatives of the Czechoslovak Society for Science and Technology for their help in the organization of the Symposium.

Bratislava, July 1987

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CHEMICAL AND BIOLOGICAL PROCESSES IN INDUSTRY

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SUMMARY

The application of biological catalysts, which represent along with isolated enzymes and enzyme systems, subcellular structures and organels with characteristic enzyme composition, and the whole cells of microorganisms and tissue cultures in preparation of inorganic and organic substances originally performed exclusively in the reaction of classical chemical synthesis, offers a perspective of new and alternative technologies. The biological processes have succesfully substituted the majority of the classical chemical processes in the food industry and in the processes where biomass or its components are starting materials. In this review the present state of the art and perspectives of development in both the biological and clasical chemical processes is described, advantages of both processes are compared and further trends in both fields are outlined.

INTRODUCTION

Large scale preparations of chemical substances based on the application of biocatalysts were already established in developed communities in ancient times. The preparation of several compounds such as ethanol, acetic acid etc. without the participation of living organisms had been unthinkable before 1845, when A. W. Kolbe performed total synthesis of acetic acid from charcoal, sulphur, chlorine and water. The social impact of the established empirical biological processes was more pronounced than that of the classical chemical production of that time. The dominating position of the raw materials rendered from biomass, gave the spontaneous biological processes superiority over the chemical technologies.

In the middle of 19th century both the microbe-based and the chemical technologies entered their more exact and antonomous period of development. The autonomous character of both conceptions reflected the famous dispute between L. Pasteur and J. von Liebig with regard to the reality underlying the phenomena of microbe based processes. In Pasteur's era of biological processes (1865-1940) the production of ethanol, propanol, 2-propanol, buthanol, 2,3-buthandiol. acetone, formic, acetic, propionic, buthyric, lactic, oxalic, succinic, citric, gluconic cids etc. was introduced. In the meantime chemical processes found their priority in the utilization of fossil resources of raw materials such as coal, petroil and natural gas. On the basis of these low entropy fossil resources the axis of chemicals - fuels was strengthened.

These fossils-based technologies fulfilled all the technical and economic requirements imposed by the industrial society and adopted by the post-industrial society in the supplying of cheap chemicals and fuels. These societies, having been built up and sustained under this prerogative of internal peace and prosperity, accept social welfare as a low entropy state being defrayed by the increase of global entropy. The accumulation of cheap chemicals in large quantities in the post-Liebig's era of chemical processes enabled the establisment of such industrial branches as the production of agrochemicals, rubber and plastics, explosives, dyes, food additives etc. and several successful break-throughs into the original domain of the microbes-based productions (synthetic ethanol, acetic acid etc.). The fossils-based industry, particulary petrochemical, was at that period of time developed to such qualitative standard and to such an extent that one could not expect a further improvement of these technologies through the use of biocatalysts.

On the other hand, the socio-economic position of the microbes--based biochemicals, proven in the combat against dangerous infectious diseases, was further strenghtened, and the era of antibiotics and vaccines began. These technologies were designed, however, for water solutions only; results obtained for processing in a gaseous state or in organic solvents were of marginal importance for the protagonists of the microbes-based technologies of that time. Expectations imposed on the chemical industry at the time of the political reorganization of the world in the thirties were expressed through the slogan "Chemistry instead of colonies" and the chemical industry of that time fulfilled these expectations offering a broad spectrum of chemical products as substitutes for those already imported from dependencies. At the beginning of the forties a solution of the problems connected with the large scale aseptic processes enabled the industrial production of antibiotics and vaccines. First experiences with the application of antibiotics showed, however, a close link between the effects of the compounds on their environment and the alterations of their properties through environmental effects due to the adopted resistance of microbes in the opposite direction. It was realized however that there are further environmental limitations for the development of both the microbes-based and the classical chemical technologies. The problem of resistance to antibiotics have been solved in two ways

a) the typically biological one, such as searching for new antibiotics and their producers, where resistance had not yet been developed,

b) chemical modification of the antibiotics with a developed resistance of microorganisms, resulting in new products, semi-synthetic antibiotics with high antimicrobial efficiency.

It was not however the only limitation to be found. Problems with pollution of the biosphere showed that there is not enough space for the deposit of both industrial and communal waste-products. Problems concerning the limited resources of the fossils were further stressed in the seventies. At that time, despite all the disadvantages characterized in the large scale technologies based on the biomass resources, the attention of chemists switched to biological processes and/or biological raw materials for the manufacture of organic chemicals because of the increaing prices of fossils.

In the following post-antibiotic era (1960-1975). when the industrial production of amino acids and single cell proteins was established, knowledge of physiology, enzymology, genetics and structure components of the living cells as well as first-hand experiences with the economy and ecological impacts of these processes was coordineted. This new inter-disciplinary outlook, with its roots in a new philosophy and noetics, stressing the role of living organisms in the eco-system and the responsibility of human beings in respect to such questions as the preservation of life on Earth, and with its apologists in the arts, has been projected among technical and natural sciences as a new research and production field known as biotechnology.

The introduction of immobilized biocatalysts (enzymes, organels and cells) radically improved both the technical performance of the process and its economy. Chemists approached this field through studies on mimics of biocatalysts and directed their activities towards the preparation of biochemicals - chemical synthesis of nucleotides and their derivatives, fragments of nucleic acids, amino acids, their analogues and peptides, lipids, mono- and oligosaccharides.

The concentrated activity of biochemists, chemists and research workers of other fields brought about an era of new biotechnologies

(1975) overwhelming the activities in the field of enzyme engineering, technologies of the recombinant DNA and the more precise knowledge of bioprocessing. The recent results gained in the field of mathematical modelling of biological processes have made the biotechnologies equal to the established chemical processes in their expressive values. Successful research in the field of application of biocatalysts in the organic solvents, in the phase boundary of the solid state substrates and in gaseous milieu made the biocatalysts competitive alternatives to those of classical chemical processes. Therefore, if the question arises as to how to implant biocatalysts into already established chemical processes e.g. into petroil refineries the reply might not be so speculative at present as it seemed to be some years ago. Similarly, more experience and practical results have been obtained with chemically synthetized oligonucleotides, opening the way to alternatives in the cell metabolism. The main aim of this review is to present the achievments and movements on both sides of the hypothetical limit-line separating the two fields i.e. chemical and biological processes, their mutual influence and their creative abilities in solving the problems of the chemical industry at the present time and in the future. In the case of the biological processes, attention is paid to the reactions of immobilized biocatalysts as well as to those of native cells carrying out their physiological processes for tehcnological purposes.

I. The elemental processes

The photoautotrophic organisms utilizing light as an energy source and CO_2 as an elemental source of carbon and the photoheterotrophic organisms utilizing different organic compounds as well as light can participate in the proccessing of elements such as hydrogen and oxygen. Biophotolysis of water for hydrogen production was first described by Krampitz (ref. 1) in 1972 in experiments with the illuminated mixture of spinach chloroplasts, methyl viologen and Escherichia coli hydrogenase. Similarly, hydrogen evolution was observed by Benemann et al. (ref. 2) a year later from the illuminated mixture of spinach chloroplasts, ferredoxin and Clostridium kluyveri hydrogenase. In this reaction the electrons stemming from the water splitting at photosystem II are transported to photosystem I to reduce the ferredoxin (or methyl viologen); the reduced carrier in turn donates the electron to hydrogenase which converts the protons in medium to molecular hydrogen (Fig. 1, ref. 3).