Edited by
A. Liese, K. Seelbach, C. Wandrey

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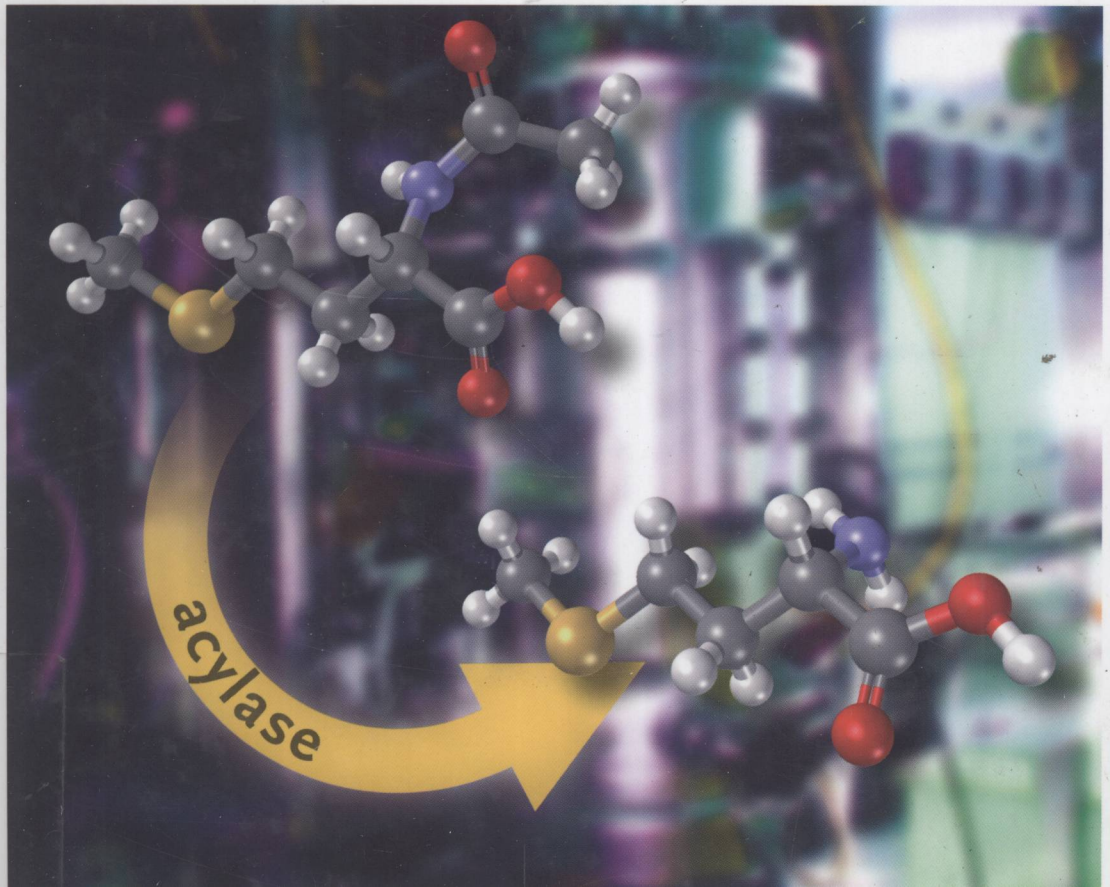
Industrial Biotransformations

Second, Completely Revised and Extended Edition



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Andreas Liese, Karsten Seelbach, Christian Wandrey

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Preface to the first edition

The main incentive in writing this book was to gather information on one-step biotransformations that are of industrial importance. With this collection, we want to illustrate that more enzyme-catalyzed processes have gained practical significance than their potential users are conscious of. There is still a prejudice that biotransformations are only needed in cases where classical chemical synthesis fails. Even the conviction that the respective biocatalysts are not available and, if so, then too expensive, unstable and only functional in water, still seems to be widespread. We hope that this collection of industrial biotransformations will in future influence decision-making of synthesis development in such a way that it might lead to considering the possible incorporation of a biotransformation step in a scheme of synthesis.

We therefore took great pains in explicitly describing the substrates, the catalyst, the product and as much of the reaction conditions as possible of the processes mentioned. Wherever flow schemes were available for publication or could be generated from the reaction details, this was done. Details of some process parameters are still incomplete, since such information is only sparingly available. We are nevertheless convinced that the details are sufficient to convey a feeling for the process parameters. Finally, the use of the products is described and a few process-relevant references are made.

We would go beyond the scope of this foreword, should we attempt to thank all those who were kind enough to supply us with examples. Of course, we only published openly available results (including the patent literature) or used personally conveyed results with the consent of the respective authors. We are aware of the fact that far more processes exist and that by the time the book is published, many process details will be outdated. Nonetheless, we believe that this compilation with its overview character will serve the above-mentioned purpose. This awareness could be augmented if the reader, using his or her experience, would take the trouble of filling out the printed worksheet at the end of this book with suggestions that could lead to an improvement of a given process or the incorporation of a further industrial process into the collection.

Requesting our industrial partners to make process schemes and parameters more accessible did not please them very much. Even so, we are asking our partners once again to disclose more information than they have done in the past. In

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many instances, far more knowledge of industrial processes has been gained than is publicly available. Our objective is to be able to make use of these "well known secrets" as well. We would like to express our gratitude to all those who supplied us with information in a progress-conducive manner. Thanks also go to those who did not reject our requests completely and at least supplied us with a photograph in compensation for the actually requested information.

The book begins with a short historical overview of industrial biotransformations. Since the process order of the compilation is in accordance with the enzyme nomenclature system, the latter is described in more detail. We also include a chapter on reaction engineering to enable an easier evaluation of the processes. The main part of the book, as you would expect, is the compilation of the industrial biotransformations. The comprehensive index will allow a facile search for substrates, enzymes and products.

We sincerely hope that this book will be of assistance in the academic as well as the industrial field, when one wants to get an insight into industrial biotransformations. We would be very thankful to receive any correction suggestions or further comments and contributions. At least we hope to experience a trigger effect that would make it worth while for the readership, the authors and the editors to have a second edition succeeding the first.

We are indebted to several coworkers for screening literature and compiling data, especially to Jürgen Haberland, Doris Hahn, Marianne Hess, Wolfgang Lanters, Monika Lauer, Christian Litterscheid, Nagaraj Rao, Durda Vasic-Racki, Murillo Villela Filho, Philomena Volkmann and Andrea Weckbecker.

We thank especially Uta Seelbach for drawing most of the figures during long nights, as well as Nagaraj Rao and the "enzyme group" (Nils Brinkmann, Lasse Greiner, Jürgen Haberland, Christoph Hoh, David Kihumbu, Stephan Laue, Thomas Stillger and Murillo Villela Filho).

And last but not least we thank our families for their support and tolerance during the time that we invested in our so called 'book project'.

Preface to the second edition

After more than five years since the first edition of "Industrial Biotransformations" many new examples have become industrially relevant, others have lost importance. Therefore we had to enlarge the chapter "Processes" by 20%. If new information about the processes of the first edition was available, this information was incorporated. All processes were checked with respect to the literature (including patent literature). We have included all the valuable corrections suggestions or further comments and contributions of many readers. This might perhaps be of great importance for the reader of the second edition. Expecting that a first edition could not be perfect, we stated in the preface to the first edition: "We would be very thankful to receive any correction suggestions or further comments and contributions. At least we hope to experience a trigger effect that would make it worthwhile for the readership, the authors and the editors to have a second edition succeeding the first." We were astonished how carefully many readers checked the information given. So the reader of the second edition will not only have an enlarged chapter "Processes", but also an updated version with – me must admit – many useful corrections. The best criticism will be given by an experienced reader. We hope very much that the "old" and the "new" readers will realize that the second edition is more than a remake of the first edition.

Since the first edition was sold out earlier than we had expected, the publisher found it scientifically – and economically – more reasonable to have a second edition than to have a reprint of the first edition. Finally after all the additional work was done we agreed with the publisher. Perhaps it is worth to be mentioned that in the meantime also the first Chinese edition appeared.

The focus of the book is still the chapter "Processes". Nevertheless all the other chapters were carefully reevaluated. In the chapter "History of Industrial Biotransformations" we included a new part "History of Biochemical Engineering".

Entirely new is the chapter "Retrosynthetic Biocatalysis". The basic idea comes from classical organic chemistry, where a complex chemical structure is reduced to building blocks, which might even be commercially available. Similarly, one can find out which easily available building blocks can be used for industrial biotransformations. We hope that the reader will find this concept useful. Especially we hope that the classical organic chemistry becomes more part of biotechnology this way.

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Entirely new is the chapter "Optimization of Industrial Enzymes by Molecular Engineering". The field of technical evolution of enzymes has become so important that we think it is justified to have a chapter devoted to the interesting and relevant findings in this field. There is no longer an "excuse" that there is no sufficiently stable, selective and active enzyme for a desired reaction. Technical evolution of enzymes has become similarly important as screening of enzymes from the environment. The chapter "Basics of Bioreaction Engineering" has been carefully checked and hopefully improved due to many valuable suggestions of the readers. We hope that bioreaction engineering will be understood as of equal importance for industrial biotransformations as enzyme engineering.

An additional short chapter is entitled "Quantitative Analysis of Industrial Biotransformation". Here the reader can find some quantitative information about the fact that it is a prejudice to believe that only hydrolases in water are useful for industrial biotransformations. Redox reactions and C-C-bond formations even in organic solvents or biphasic systems are also industrially relevant today.

Our original understanding of "Industrial Biotransformations" was a one step reaction of industrial relevance. This definition might become less clear in the future, because also two or three step biotransformations are or might be included. So it will become more and more difficult to distinguish an industrial biotransformation from a fermentation process. This is especially true in the age of "designer bugs", where a microorganism is first grown and than used as a more or less "non-growing catalyst" for industrial biotransformation. But we should not bother too much with definitions. Our aim was and is to show that biotransformations are of great importance in the academic and industrial fields. Since the first edition the field of "White Biotechnology" (formally known as Industrial Microbiology) has developed a lot. A quantative understanding of complex microbial systems by means of the "polyomics" techniques (genomics, proteomics, and metabolomics) has improved so much that we can expect recombinant pathways for industrial biotransformations used in a biocatalysis under non-natural conditions. We can foresee that the technical evolution not only of biocatalyst but also of bioprocesses will lead to many more industrial biotransformations. Thus, sooner or later we expect the burden/pleasure that we will have to prepare a third edition of "Industrial Biotransformations".

We would like to ask the reader again to help us with "correction suggestions or further comments and contributions". The best compensation for all the work the author of a book can get is the feeling that it is read by colleagues who understand the subject.

Last but not least we would like to mention in addition to the many coworkers who have contributed to the first edition, now the additional valuable contributions of many more who helped us to prepare the second edition, especially Karl-Heinz Drauz, Kurt Faber, Katja Goldberg, Udo Kragl, Peter Stahmann, Trevor Laird, John Villadsen, Ulrike Zimmermann. Especially we thank our families for their support during the time that we invested in the second edition of this book.

Dezember 2005

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Contents

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1
History of Industrial Biotransformations – Dreams and Realities

Durda Vasic-Racki

Throughout the history of mankind, microorganisms have been of enormous social and economic importance. Without even being aware of their existence, very early on in history man was using them in the production of food and beverages. The Sumerians and Babylonians were practising the brewing of beer before 6000 BC, references to wine making can be found in the Book of Genesis and the Egyptians used yeast for baking bread. However, knowledge of the production of chemicals such as alcohols and organic acids through fermentation is relatively recent and the first reports in the literature only appeared in the second half of the 19th century. Lactic acid was probably the first optically active compound to be produced industrially by fermentation. This was accomplished in the USA in 1880 [1]. In 1921, Chapman reviewed a number of early industrial fermentation processes for organic chemicals [2].

In the course of time, it was discovered that microorganisms could modify certain compounds by simple, chemically well defined reactions, which were further catalyzed by enzymes. Nowadays, these processes are called "biotransformations". The essential difference between fermentation and biotransformation is that there are several catalytic steps between the substrate and the product in a fermentation while there are only one or two in a biotransformation. The distinction is also in the fact that the chemical structures of the substrate and the product resemble one another in a biotransformation, but not necessarily in a fermentation.

1.1
From the "Flower of Vinegar" to Recombinant *E. Coli* – The History of Microbial Biotransformations

The story of microbial biotransformations is closely associated with vinegar production which dates back to around 2000 years BC.

Vinegar production is perhaps the oldest and best known example of microbial oxidation, which can illustrate some of the important developments in the field of biotransformations by living cells (Fig. 1.1).

Since ancient times, man has wanted to see things that are far smaller than can be perceived with the naked eye. In the 16th century this led to the construction of a magnifier

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Fig. 1.1 Vinegar production.

consisiting of a single convex lens, and this, in turn, led eventually to the development of the microscope (Fig. 1.2). Antony von Leeuwenhoek (1632–1723) became the first person, or microscopist [3], to make and use a real microscope. He described microorganisms including bacteria, algae and protozoa in fresh water (Fig. 1.3). In fact he constructed a total of 400 microscopes during his lifetime. Subsequently, the compound microscope system was invented in the 17th century. This type of microscope, incorporating more than one lens, has made tremendous contributions to the progress of science. Using this microscope Hooke (1635–1703) discovered the fact that living things are composed of cells, and later on Pasteur, among others, discovered yeast fungus. The microscope has possibly had a greater impact on the development of knowledge than any other scientific instrument in history [4]. The discovery of new microscopic life was the starting point for experimental biology as a basis for the development of the biotransformations.

A prototype bioreactor with immobilized bacteria has been known in France since the 17th century. The oldest bioreactor to use immobilized living microorganisms, a so-called generator, was developed in 1823 [5, 6]. Even today, acetic acid is still known as "vinegar" if it is obtained by oxidative fermentation of ethanol-containing solutions by acetic acid bacteria [7].

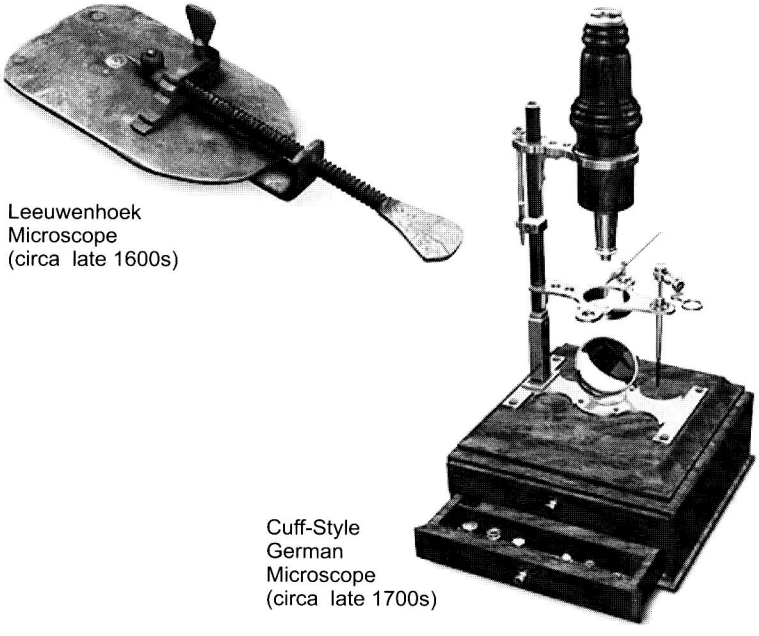


Fig. 1.2 Historical microscopes (photographs courtesy of Michael W. Davidson).

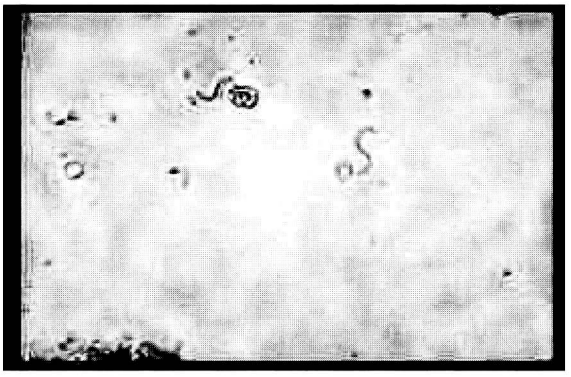
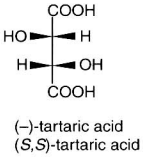


Fig. 1.3 Spiral bacteria (photograph courtesy of Michael W. Davidson).

In 1858, Pasteur [8] was the first to demonstrate the microbial resolution of tartaric acid. He performed fermentation of the ammonium salt of racemic tartaric acid, mediated by the mold *Penicillium glaucum*. The fermentation yielded (–)-tartaric acid (Fig. 1.4).



(–)-tartaric acid
(*S*,*S*)-tartaric acid

Fig. 1.4 Pasteur's product of the first resolution reaction.

This was also the first time that a method was used where the microorganisms degraded one enantiomer of the racemate while leaving the other untouched.

In 1862, Pasteur [9] investigated the conversion of alcohol into vinegar and concluded that pellicle, which he called "the flower of vinegar", "served as a method of transport for the oxygen in air to a multitude of organic substances".

In 1886 Brown confirmed Pasteur's findings and gave the causative agent in vinegar production the name *Bacterium xylinum*. He also found that it could oxidize propanol to propionic acid and mannitol to fructose (Fig. 1.5) [10].

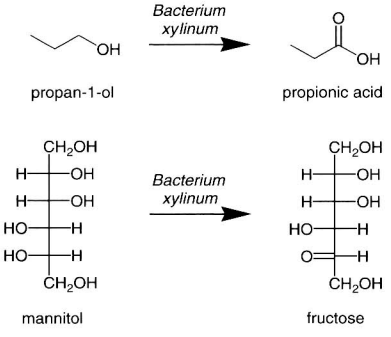


Fig. 1.5 Reactions catalyzed by *Bacterium xylinum*, the vinegar biocatalyst.