A. Liese, K. Seelbach, C. Wandrey

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This book was carefully produced. Nevertheless, authors and publisher do not warrant the information contained therein to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

The cover illustration shows the production plant of l-methionine. The picture was supplied by Professor W. Leuchtenberger, Degussa-Hüls AG. In the foreground the conversion of N-acetyll-methionine with acylase (EC 3.5.1.14) to l-methionine is shown.
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1 Introduction

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 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’.
2 History of Industrial Biotransformations – Dreams and Realities

DURDA VASIC-RACKI
Faculty of Chemical Engineering and Technology
University of Zagreb
HR-10000 Zagreb, Croatia

Throughout the history of mankind, microorganisms have been of tremendous social and economic importance. Without even being aware of their existence, man used them in the production of food and beverages already very early in history. Sumerians and Babylonians practised beer brewing before 6000 B.C., references to wine making can be found in the Book of Genesis, and Egyptians used yeast for baking bread. However, the knowledge of the production of chemicals such as alcohols and organic acids by fermentation is relatively recent and the first reports in the literature appeared only in the second half of the 19th century. Lactic acid was probably the first optically active compound to be produced industrially by fermentation. It 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 substrate and product in fermentation while there are only one or two in 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.

2.1 From the “flower of vinegar” to the recombinant E. coli – The history of microbial biotransformations

The story of microbial biotransformations is closely connected with vinegar production which dates back to some 2000 years B.C.

Vinegar production is perhaps the oldest and best known example of microbial oxidation which may illustrate some of the important developments in the field of biotransformations by living cells (figure 1).
A prototype bioreactor with immobilized bacteria has been known in France since the 17th century. The oldest bioreactor using immobilized living microorganisms, a so-called generator, was developed in 1823 [3,4]. Even today, acetic acid is still known as “vinegar” if it is obtained by oxidative fermentation of ethanol-containing solutions by acetic acid bacteria [5].

In 1858, Pasteur [6] 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 (figure 2).

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

In 1862, Pasteur [7] investigated the conversion of alcohol to vinegar and concluded that the pellicle, which he called “the flower of vinegar”, “serves as a transport of air oxygen to a multitude of organic substances”.

In 1886, Brown confirmed Pasteur’s findings and named the causative agent in vinegar production as Bacterium xylinum. He also found that it could oxidize propanol to propionic acid and mannitol to fructose (figure 3) [8].
In 1897, Buchner [9] reported that cell-free extracts prepared by grinding yeast cells with sand could carry out alcoholic fermentation reactions in the absence of living cells. This initiated the usage of resting cells for biotransformations.

Neuberg and Hirsch [10] discovered in 1921 that the condensation of benzaldehyde with acetaldehyde in the presence of yeast forms optically active 1-hydroxy-1-phenyl-2-propanone (figure 4).

![Chemical reaction diagram](image)

1 = benzaldehyde  
2 = 2-oxo-propionic acid  
3 = 1-hydroxy-1-phenylpropan-2-one  
4 = 2-methylamino-1-phenylpropan-1-ol

**Fig. 4** L-Ephedrine production.

The obtained compound was further chemically converted into D-(-)-ephedrine by Knoll AG, Ludwigshafen, Germany in 1930 (figure 5) [11].
The bacterium *Acetobacter suboxydans* was isolated in 1923 [12]. Its ability to carry out limited oxidation was used in a highly efficient preparation of L-sorbose from D-sorbitol (figure 6).

Fig. 6 Reichstein-Grüssner synthesis of vitamin C (L-ascorbic acid).

L-Sorbose became important in the mid-1930's as an intermediate in the Reichstein-Grüssner synthesis of L-ascorbic acid [13].

In 1953, Peterson at al. [14] reported that *Rhizopus arrhizus* converted progesterone to 11α-hydroxyprogesterone (figure 7), which was used as an intermediate in the synthesis of cortisone.

Fig. 7 Microbial 11α-hydroxylation of progesterone.
This microbial hydroxylation simplified and considerably improved the efficiency of the multi-step chemical synthesis of corticosteroid hormones and their derivatives. Although the chemical synthesis [15] (figure 8) from deoxycholic acid that was developed at Merck, Germany, was workable, it was recognized that it was complicated and uneconomical: 31 steps were necessary to obtain 1 kg of cortisone acetate from 615 kg of deoxycholic acid. The microbial 11α-hydroxylation of progesterone quickly reduced the price of cortisone from $200 to $6 per gram. Further improvements have led to a current price of less than $1 per gram [16].

In the 1950’s, the double helix structure and the chemical nature of RNA and DNA – the genetic code of heredity – were discovered. This discovery can be regarded as one of the milestones among this century’s main scientific achievements. It led to the synthesis of recombinant DNA and gave a fillip to genetic engineering in the seventies’. Such developments quickly made the rDNA technology a part of industrial microbial transformations. Application of this technology for the production of small molecules began in 1983. Ensley et al. [17] reported on the construction of a strain of E. coli that excreted indigo, one of the oldest known dyes. They found that the entire pathway for conversion of naphthalene to salicylic acid is encoded by genes of Pseudomonas putida. These genes can be expressed in E. coli. Their results led to the unexpected finding that a subset of these genes was also responsible for the microbial production of indigo. Moreover, they showed that indigo formation was a property of the dioxygenase enzyme system that forms cis-dihydrodiols from aromatic hydrocarbons. Finally, they proposed a pathway for indigo biosynthesis in a recombinant strain of E. coli (figure 9).

Genencor International is developing a commercially competitive biosynthetic route to indigo using recombinant E. coli that can directly synthesize indigo from glucose [18]. Anderson et al. in 1985 [19] reported the construction of a metabolically engineered bacterial strain that was able to synthesize 2-keto-L-gulonic acid (figure 10), a key intermediate in the production of L-ascorbic acid (vitamin C).

BASF, Merck and Cerestar are building a 2-keto-L-ketogulonic acid plant in Krefeld, Germany. The start up of operation is scheduled for 1999. They developed a new fermentation route from sorbitol directly to the ketogulonic acid [20]. This method is probably similar to the method described in 1966 [21].

The Cetus Corporation (Berkeley, California, USA) bioprocess for converting alkenes to alkene oxides emerged in 1980 [22]. This bioprocess appeared to be very interesting, thanks to the possibility of replacing energy-consuming petrochemical processes.

There were high hopes that the development of recombinant DNA technology would speed up technological advances. Unfortunately, there is still a lot left to be done about the development and application of bioprocesses before the commercial production of low-value chemicals becomes feasible [23]. However, today even the traditional chemical companies like Dow Chemical, DuPont, Degussa-Hüls AG etc., pressurized by investors and technological advances, are trying to use microbial or enzymatic transformations in production. They are doing this to see whether natural feedstocks can bring more advantages than crude oil. One only needs to compare the cost of a barrel of oil with that of corn starch to see that the latter is quite cheaper [20].
Fig. 8 Chemical synthesis of cortisone.
2.1 From the "flower of vinegar" to the recombinant E. coli

![Chemical reactions and biological routes to indigo](image)

**Fig. 9** Comparison of chemical and biological routes to indigo.

![Biosynthesis of 2-keto-L-gulonic acid](image)

**Fig. 10** Biosynthesis of 2-keto-L-gulonic acid.

Acrylamide is one of the most important commodity chemicals. Its global consumption is about 200,000 tonnes per year. It is used in the production of various polymers for use as flocculants, additives or for petroleum recovery. In conventional synthesis, copper salts are used as catalysts in the hydration of nitriles. However, this is rather disadvantageous as the preparation of the catalysts is quite complex. Additionally, it is difficult to regenerate the used catalyst and separate and purify the formed acrylamide. Furthermore, since acrylamides are readily polymerized, their production under moderate conditions is highly desirable. In contrast to the conventional chemical process, there is no need to recover unreacted acrylonitrile in the enzymatic process, because the conversion and yield of the enzymatic hydration pro-
cess are almost 100%. The removal of the copper ions from the product is no longer necessary. Overall, the enzymatic process—being carried out below 10°C under mild reaction conditions and requiring no special energy source—proves to be simpler and more economical. The immobilized cells are used repeatedly and a very pure product is obtained. The enzymatic process, which was first implemented in 1985, is already producing about 6000 tons of acrylamide per year for Nitto [24,25]. The use of biocatalyst for the production of acrylamide may not be the first case in which biotransformation as a part of biotechnology was used in the petrochemical industry. However, it is the first successful example of the introduction of an industrial biotransformation process for the manufacture of a commodity chemical (figure 11).

![Acrylamide synthesis](attachment:image11.png)

**Fig. 11** Acrylamide synthesis.

Some representative industrial microbial transformations are listed in Table I.

<table>
<thead>
<tr>
<th>Product</th>
<th>Biocatalyst</th>
<th>Operating since</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>vinegar</td>
<td>bacteria</td>
<td>1823</td>
<td>various</td>
</tr>
<tr>
<td>L-2-methylamino-1-phenylpropan-1-ol</td>
<td>yeast</td>
<td>1930</td>
<td>Knoll AG, Germany</td>
</tr>
<tr>
<td>L-sorbose</td>
<td><em>Acetobacter suboxydans</em></td>
<td>1934</td>
<td>various</td>
</tr>
<tr>
<td>prednisolone</td>
<td><em>Arthrobacter simplex</em></td>
<td>1955</td>
<td>Schering AG, Germany</td>
</tr>
<tr>
<td>L-aspartic acid</td>
<td><em>Escherichia coli</em></td>
<td>1958</td>
<td>Tanabe Seiyaku Co., Japan</td>
</tr>
<tr>
<td>7-ADCA</td>
<td><em>Bacillus megaterium</em></td>
<td>1970</td>
<td>Asahi Chemical Industry, Japan</td>
</tr>
<tr>
<td>L-malic acid</td>
<td><em>Brevibacterium ammoniagenes</em></td>
<td>1974</td>
<td>Tanabe Seiyaku Co., Japan</td>
</tr>
<tr>
<td>D-p-hydroxyphenylglycin</td>
<td><em>Pseudomonas striata</em></td>
<td>1983</td>
<td>Kanegafuchi, Chemical Co., Japan</td>
</tr>
<tr>
<td>acrylamide</td>
<td><em>Rhodococcus sp.</em></td>
<td>1985</td>
<td>Nitto Chemical Ltd, Japan</td>
</tr>
<tr>
<td>D-aspartic acid and L-alanine</td>
<td><em>Pseudomonas dacunha</em></td>
<td>1988</td>
<td>Tanabe Seiyaku Co., Japan</td>
</tr>
<tr>
<td>2-keto-L-gulonic acid</td>
<td><em>Acetobacter sp.</em></td>
<td>1999</td>
<td>BASF, Merck, Cerestar, Germany</td>
</tr>
</tbody>
</table>
2.2 From gastric juice to Sweetzyme® – The history of enzymatic biotransformations

Enzymes were in use for thousands of years before their nature was gradually understood. No one really knows when the calf stomach was used as a catalyst for the first time in the manufacture of cheese.

As early as 1783, Spallanzani showed that gastric juice secreted by cells could digest meat in vitro. In 1836, Schwan called the active substance pepsin [26]. In 1876, Kühne (figure 12) presented a paper to the Heidelberger Natur-Historischen und Medizinischen Verein, suggesting that such non-organized ferments should be called enzymes [27]. At that time two terms were used: “organized ferment” such as cell-free yeast extract from Büchner, and “unorganized ferment” such as gastric juice secreted by cells. Today the terms “intracellular” and “extracellular” are used. Kühne also presented some interesting results from his experiments with trypsin. The word “enzyme” comes from Greek for “in yeast” or “leavened” [28].

Microorganisms synthesize numerous enzymes, each having its own function. Intracellular enzymes operate inside the cell in a protected and highly structured environment, while extracellular enzymes are secreted from the cell, thus working in the medium surrounding the microorganism.

The commercial usage of extracellular microbial enzymes started in the West around 1890, thanks to the Japanese entrepreneur Takamine. He settled down in the United States and started an enzyme factory based on Japanese technology. The principal product was called takadiastase. This was a mixture of amylolytic and proteolytic enzymes prepared by cultivation of Aspergillus oryzae. In France, Boidin and Effront developed bacterial enzymes in 1913. They found that the hay bacillus, Bacillus subtilis, produces an extremely heat-stable α-amylase when grown in still cultures on a liquid medium prepared by extraction of malt or grain [29].

In 1894, Emil Fischer [30,31] observed in his studies of sugars that the enzyme called emulsin catalyzes the hydrolysis of β-methyl-d-glucoside, while the enzyme called maltase is active towards the α-methyl-d-glucoside as substrate (figure 13). This led Fischer to suggest his famous “lock–and-key” theory of enzyme specificity, which he would describe in his own words as follows: “To use a picture, I would say that enzyme and the glucoside must fit into each other like a lock and key, in order to effect a chemical reaction on each other” [1].

In 1913, Michaelis and Menten published a theoretical consideration of enzymatic catalysis. This consideration envisaged the formation of a specific enzyme-substrate complex which further decomposed and yielded the product with the release of the enzyme. This led to the development of the famous Michaelis-Menten equation to describe the typical saturation kinetics observed with purified enzymes and single substrate reactions [32].

By 1920, about a dozen enzymes were known, none of which had been isolated [33]. Then, in 1926, Sumner [34] crystallized urease from jack bean, Canavalia ensiformis, and announced that it was a simple protein.
Ueber das Verhalten verschiedener organisirter
und sog. ungeformter Fermente.

Ueber das Trypsin (Enzym des Pankreas).

Von W. Kühne.

1876

Fig. 12 W. F. Kühne [27].
Northrop and his colleagues [26] soon supported Sumner's claim that an enzyme could be a simple protein. They isolated many proteolytic enzymes beginning with pepsin in 1930 by applying classical crystallization experiments. By the late 1940s many enzymes were available in pure form and in sufficient quantity for investigation of their chemical structure. Currently, more than 3,000 enzymes have been catalogued [35]. The ENZYME data bank contains information related to the nomenclature of enzymes [36]. The current version contains 3,705 entries. It is available through the ExPASy WWW server (http://www.expasy.ch/). Several hundreds of enzymes can be obtained commercially [37].

In 1950, there was still no evidence that a given protein had a unique amino acid sequence. Lysosyme was the first enzyme whose tertiary-structure (figure 14) was defined in 1966 with the help of X-ray crystallography [38].
Fig. 14 Stereo photographs of models of part of the lysozyme molecule [38].
Further, ribonuclease A was one of the first enzymes made on a laboratory scale by organic chemistry methods. In 1969, Gutte and Merrifield synthesized its whole sequence in 11,931 steps [39].

By 1970, the complete molecular structures of several enzymes had been established and plausible reaction mechanisms could be discussed [26].

Hill (1897) was the first to show that the biocatalysis of hydrolytic enzymes is reversible [40].

Pottevin (1906) went further and demonstrated that crude pancreatic lipase could synthesize methyl oleate from methanol and oleic acid in a largely organic reaction mixture [41].

While the first benefit for the industry from the microbiological development had come early, the investigations with isolated enzymes hardly influenced the industry at that time. Consequently, industrial enzymatic biotransformations have a much shorter history than microbial biotransformations in the production of fine chemicals.

Invertase was probably the first immobilized enzyme to be used commercially for the production of Golden Syrup by Tate & Lyle during World War II, because sulfuric acid as the preferred reagent was unavailable at that time (figure 15) [42].

![Fig. 15 Inversion of sucrose by invertase.](image)

Yeast cells were autolysed and the autolysate clarified by adjustment to pH 4.7, followed by filtration through a calcium sulphate bed and adsorption into bone char. A bone char layer containing invertase was incorporated into the bone char bed, which was already used for syrup decolorisation. The scale of operation was large, the bed of invertase-char being 60 cm deep in a 610 cm deep bed of char. The preparation was very stable since the limiting factor was microbial contamination or loss of decolorising power rather than the loss of enzymatic activity. The process was cost-effective but the product did not have the flavor quality of the acid-hydrolysed material. This is the reason why the immobilized enzyme was abandoned once the acid became available again [42].

Industrial processes for l-amino acid production based on the batch use of soluble aminoacylase were already in use in 1954. However, like many batch processes with soluble enzymes, they had their disadvantages such as higher labor costs, complicated product separation, low yields, high enzyme costs and non-reusability of enzyme. During the mid-1960s the Tanabe Seiyaku Co. of Japan was trying to overcome these problems by using immobilized aminoacylases. In 1969, they started the industrial production of l-methionine by aminoacylase immobilized on DEAE-Sephadex in a packed bed reactor (figure 16). This was the first full scale industrial use of an immobilized enzyme. The most important advantages are the relative simplicity and ease of control [44].
Fig. 16 L-Amino acid production catalyzed by aminoacylase.

In a membrane reactor system developed at Degussa-Hüls AG in Germany in 1980 [45], native enzymes, either pure or of technical grade, are used in homogeneous solution for the large scale production of enantiomerically pure L-amino acids (figure 17).

A membrane reactor is particularly well suited for cofactor-dependent enzyme reactions, especially if the cofactor is regenerated by another enzyme reaction and retained by the membrane in modified form [46]. There are several advantages of carrying out biocatalysis in membrane reactors over heterogeneous enzymatic catalysis: there are no mass transfer limitations, enzyme deactivation can be compensated for by adding soluble enzyme and the reactors can be kept sterile more easily than immobilized enzyme systems. The product is mostly pyrogen free (major advantage for the production of pharmaceuticals), because the prod-
uct stream passes through an ultrafiltration membrane. Scale-up of membrane reactors is simple because large units with increased surface area can be created by combining several modules.

The enzymatic isomerization of glucose to fructose (figure 18) represents the largest use of an immobilized enzyme in the manufacture of fine chemicals.

Fig. 18 Isomerization of glucose to fructose.

High-fructose corn syrup HFCS has grown to become a large-volume biotransformation product [47]. While sucrose is sweet, fructose is approximately 1.5 times sweeter and consequently high quality invert syrups (i.e. hydrolyzed sucrose) may be produced. Invert syrups contain glucose and fructose in a 1:1 ratio. However, the food industry needed a long time to become acquainted with the glucose isomerase potential to produce high quality fructose syrups from glucose. Again, the Japanese were the first to employ soluble glucose isomerase to produce high quality fructose syrups in 1966. At the beginning of 1967, Clinton Corn Processing Company, Iowa, USA, was the first company to manufacture enzymatically produced fructose corn syrup [47].The glucose-isomerase catalyzed reversible reaction gave a product containing about 42% of fructose, 50% of glucose and 8% of other sugars. Due to various reasons, economic viability being the more important among them, the first commercial production of fructose syrups using glucose isomerase immobilized on a cellulose ion-exchange polymer in a packed bed reactor plant started only in 1974. It was initiated by Clinton Corn Processing [44]. In 1976, Kato was the first company in Japan to manufacture HFCS in a continuous process as opposed to a batch process. In 1984, it became the first company to isolate crystalline fructose produced in this process by using an aqueous separation technique.

The glucose isomerase Sweetzyme T, produced by Novo, Denmark is used in the starch processing industry in the production of high fructose syrup. The key to its long life is immobilization. The enzyme is chemically bound to a carrier, making the particles too large to run out through the sieve at the bottom of the isomerization columns. Sweetzyme T is packed into columns where it is used to convert glucose into fructose. The record for the longest lifetime of a column is 687 days, held by a Japanese company called Kato Kagaku in Kohwa near Nagoya. The reaction conditions are pH 7.5 and $T = 55^\circ C$. Though enzyme activity is reduced at this temperature, its stability and productivity are considerably improved [48].

The engineers from Kato used to say: "The better the substrate you put in, the better the results you get out". Each column at Kato contains 1,800 kg of Sweetzyme T. The column needs to be changed when the flow rate decreases to about 10% of the initial value. Sweetzyme T displays a linear decay curve under steady state operating conditions. With regard to productivity, the yield from the record-
The breaking column was 12,000 kg of fructose syrup (containing 42% fructose) (dry substance)/kg of Sweetzyme T. The normal column productivity was 8,000–10,000 kg/kg enzyme. The 687 days’ record for Sweetzyme T is also a world record in the starch industry [48] (figure 19).

![Stability graph]

**Fig. 19** Improved biocatalyst stability by biocatalyst engineering at Novo.

“Central del Latte” of Milan, Italy, was the first company which commercially hydrolyzed milk lactose with immobilized lactase using SNAMprogetti technology [49]. An industrial plant with a capacity of 10 tons per day is situated in Milan. The entrapped enzyme is lactase obtained from yeast and the reaction is performed batchwise at low temperature. Lactase hydrolyses lactose, a sugar with poor solubility properties and a relatively low degree of sweetness, to glucose and galactose (figure 20).

![Lactose hydrolysis]

**Fig. 20** β-Galactosidase catalyzed hydrolysis of lactose to galactose and glucose.

After the processed milk reaches the desired degree of hydrolysis of lactose, it is separated from the enzyme fibers, sterilized, and sent for packing and distribution. SNAMprogetti’s process enables the manufacture of a high-quality dietary milk at low cost. This milk has a remarkable digestive tolerance, pleasant sweetness, unaltered organoleptic properties, and good shelf-life. It does not contain foreign matter. The industrial plant is shown in figure 21.
Penicillin G, present in *Penicillium notatum* and discovered by Fleming in 1929, revolutionized chemotherapy against pathogenic microorganisms. Today, β-lactam antibiotics such as penicillins and cephalosporins are very widely used. Thousands of semisynthetic β-lactam antibiotics are being synthesized to find more effective compounds. Most of these compounds are prepared from 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA) and 7-amino-desacetoxyccephalosporanic acid (7-ADCA).

At present, 6-APA is mainly produced either by chemical deacylation or by enzymatic deacylation using penicillin amidase from penicillin G or V. This process, which exemplifies the best known usage of an immobilized enzyme in the pharmaceutical industry, is being used since around 1973 (figure 22). Several chemical steps are replaced by a single enzymatic reaction. Organic solvents, the use of low temperature (−40°C) and the need for absolutely anhydrous conditions, which made the process difficult and expensive, were no longer necessary in the enzymatic process [50].
For many years enzymatic 7-ACA production was nothing but a dream. This changed in 1979, when Toyo Jozo, Japan, in collaboration with Asahi Chemical Industry, also Japan, developed and succeeded in the industrial production of 7-ACA by a chemoenzymatic two-step process starting from cephalosporin C (figure 23):

![Chemical Reaction](image)

The chemical process requires highly purified cephalosporin C as raw material. A number of complicated reaction steps are carried out at -40°C to -60°C, and the reaction time is long. Furthermore, hazardous reagents, such as phosphorous pentachloride, nitrosyl chloride and pyridine are used in this process. The removal of such reagents causes significant problems. Therefore, the development of an enzymatic process was a dream for a long time. In the enzymatic process, liberated glutaric acid reduces the pH and inhibits the glutaryl-7-ACA amidase, the enzyme that catalyzes the deacylation of cephalosporin C. Because of this change in pH the reaction rate is decreased, requiring strict pH control during the reaction process. For these reasons, a recirculation bioreactor with immobilized glutaryl-7-ACA amidase and an automatic pH controller were designed for the 7-ACA production. The bioreactor for industrial 7-ACA production is shown in figures 24 and 25. The process has been in operation at Asahi Chemical Industry since 1973. It is reported that about 90 tons of 7-ACA are thus produced annually [51].
2.2 From gastric juice to Sweetzyme™ – The history of enzymatic biotransformations

Fig. 24 Flow scheme for the production of 7-ACA. Production carried out at Asahi Chemical Industry. ($E_1 = \text{d-aminoacid oxidase;} E_2 = \text{glutaryl amidase}$).
Four technological advances, having major impact on enzymatic biotransformations, were required for the acceptance of enzymes as ‘alternative catalysts’ in industry [52].

The first technological advance was the development of large-scale techniques for the release of enzymes from the interior of microorganisms [53]. Although the majority of industrial purification procedures are based on the same principles as those employed at laboratory scale, the factors under consideration while devising industrial scale purification regimes are somewhat different. When isolating enzymes on an industrial scale for commercial purposes, a prime consideration has to be the cost of production in relation to the value of the end prod-