1
Introduction to Biocatalysis

Summary

Over the last 20 years, many reservations with respect to biocatalysis have been voiced, contending that: (i) enzymes only feature limited substrate specificity; (ii) there is only limited availability of enzymes; (iii) only a limited number of enzymes exist; (iv) protein catalyst stability is limited; (v) enzyme reactions are saddled with limited space–time yield; and (vi) enzymes require complicated cosubstrates such as cofactors.

Driven by the discovery of many novel enzymes, by recombinant DNA technology which allows both more efficient production and targeted or combinatorial alterations of individual enzymes, and by process development towards higher stability and volumetric productivity, synthesis routes in which one or all of the steps are biocatalytic have advanced dramatically in recent years. Design rules for improved biocatalysts are increasingly precise and easy to use.

Biocatalysts do not operate by different scientific principles from organic catalysts. The existence of a multitude of enzyme models including oligopeptidic or polypeptidic catalysts proves that all enzyme action can be explained by rational chemical and physical principles. However, enzymes can create unusual and superior reaction conditions such as extremely low pK_a values or a high positive potential for a redox metal ion. Enzymes increasingly have been found to catalyze almost any reaction of organic chemistry.

Biotechnology and biocatalysis differ from conventional processes not only by featuring a different type of catalyst; they also constitute a new technology base. The *raw materials base* of a biologically-based process is built on sugar, lignin, or animal or plant wastes; in biotechnology, unit operations such as membrane processes, chromatography, or biocatalysis are prevalent, and the product range of biotechnological processes often encompasses chiral molecules or biopolymers such as proteins, nucleic acids or carbohydrates.

Cost and margin pressure from less expensive competitors and operation with emphasis on a clean (or less polluted) environment are two major developments. Fewer processing steps, with higher yields at each step, lower material and energy costs, and less waste are the goals. Biotechnology and biocatalysis often offer unique technology options and solutions, they act as *enabling technologies*; in other cases, biocatalysis has to outperform competing technologies to gain access. In the phar-

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ISBN: 3-527-30344-8

maceutical industry, the reason for the drive for enantiomeric purity is that the vast majority of novel drugs are chiral targets, favoring biocatalysis as the technology with the best selectivity performance.

Biocatalytic processes increasingly penetrate the chemical industry. In a recent study, 134 industrial-scale biotransformations, on a scale of > 100 kg with whole cells or enzymes starting from a precursor other than a C-source, were analyzed. Hydrolases (44%), followed by oxido-reductases (30%), dominate industrial biocatalytic applications. Average performance data for fine chemicals (not pharmaceuticals) applications are 78% yield, a final product concentration of 108 g L^{-1}, and a volumetric productivity of 372 g $(L \cdot d)^{-1}$.

1.1 Overview:The Status of Biocatalysis at the Turn of the 21st Century

1.1.1 State of Acceptance of Biocatalysis

Over the last 20 years, many reservations with respect to biocatalysis have been voiced. The critics, often focusing on the drawbacks, have contended that

- enzymes only feature limited substrate specificity,
- there is only limited availability of enzymes,
- only a limited number of enzymes exist,
- protein catalyst stability is limited,
- enzyme reactions are saddled with limited space–time yield, and
- enzymes require complicated co-substrates such as cofactors.

The renaissance of biocatalysis, supported by the advent of recombinant DNA, is only about 20 years old. Recently, several publications have appeared which deal specifically with the attitudes listed above (Rozzell, 1999; Bommarius, 2001; Rasor, 2001). Most of the points above can either be refuted or they can be levied against any novel catalytic technology; the situation with some competing technologies such as chiral homogeneous catalysts is similar to that with enzymes (Chapters 18 and 20).

- *Enzymes only feature limited substrate specificity*. Often, enzymes designed to convert small molecules such as hydrogen peroxide, urea, fumaric acid, or L-aspartic acid feature extremely narrow substrate specificity; the corresponding enzymes catalase, urease, fumarase or aspartase, and L-aspartate decarboxylase take either few other substrates, such as alkyl peroxides in the case of catalase, or no other substrate, such as urease, which only converts urea. On the other hand, very large enzymes acting as multi-enzyme complexes such as nonribosomal peptide synthetases (NRPSs) (Kleinkauf, 1996) are often highly specific. Ordinary-sized enzymes working on medium-sized substrates, however, in most cases feature broad substrate specificity, a fact already noted by Rasor and Voss (Rasor, 2001).

- *There is only limited availability of enzymes.* Until very recently, limited availability of enzymes was indeed a major problem. About ten years ago, with 3196 different enzymes already listed in Enzyme Nomenclature (Moss, 1992), only about 50 enzymes were fully characterized and only about a dozen enzymes available commercially on a regular basis. However, recombinant DNA technology, discovered in 1978 by Cohen and Boyer (Stanford University, Palo Alto, CA, USA), over the next 20 years allowed enzymes to be produced much more efficiently, in higher purity, and more inexpensively (Baneyx, 1999), so that today a multitude of enzymes are available not only from established suppliers such as Sigma–Aldrich–Fluka (Milwaukee, WI, USA), E. Merck (Darmstadt, Germany), Mercian (Tokyo, Japan), or Roche Diagnostics (Mannheim, Germany) but increasingly also from smaller, more focused suppliers such as Biocatalytics (Pasadena, CA, USA) or Jülich Fine Chemicals (Jülich, Germany). The argument of unavailability or scarcity will be less and less justified in the future.

- *Only a limited number of enzymes exist.* This criticism, while depending on the observer's position, is indeed a drawback at the moment. Although enzymes have been found for every conceivable organic chemical reaction except the hetero-Cope rearrangement (Table 1.4, below), there are enzymes sought for many more reactions than there are enzymes available. If enzymes were inferior catalysts this situation would not arise, of course. In fact, enzymes are often superior catalysts (see the next section), so superior is fact, that the community seeks plenty more of them. Chapter 3 treats the discovery of novel enzymes, whereas Chapters 10 and 11 cover improvement of existing enzymes through rational (protein engineering) and combinatorial random mutagenesis (directed evolution).

- *Protein catalyst stability is limited.* This is one of major drawbacks of enzymes. They commonly require temperatures around ambient to perform (15–50°C), pH values around neutral (pH 5–9), and aqueous media. In addition, any number of system components or features such as salts, inhibitors, liquid–gas or liquid–solid interfaces, or mechanical stress can slow down or deactivate enzymes. Under almost any condition, native proteins, with their Gibbs free enthalpy of stability of just a few kilojoules per mole, are never far away from instability. In this book, we cover inhibitors (Chapter 5, Section 5.3) or impeding system parameters (Chapter 17) and successful attempts at broadening the choice of solvents (Chapter 12).

- *Enzyme reactions are saddled with limited space–time yield.* The notion that biocatalysts are slow catalysts is false. Slow catalysts, applied at low concentrations, certainly lead to low space–time yields. However, optimized syntheses not only produce very good selectivities or total turnover numbers but also satisfactory to excellent space–time yields. Examples with such good s.t.y. values are
  - in commodity biochemicals, the synthesis of L-aspartate from fumaric acid and ammonia with space–time-yields of up to 60 kg $(L \cdot d)^{-1}$ (Rozzell, 1999), and

– in advanced pharmaceutical intermediates, kinetically-controlled peptide synthesis to kyotorphin (Tyr-Arg) catalyzed by α-chymotrypsin from maleyl-L-Tyr-OEt and Arg-OEt, employing a highly soluble protecting group at the electrophile (Fischer, 1994). Space–time-yields of 1.34 kg $(L \cdot d)^{-1}$ have been achieved.

The question of high volumetric productivity is coupled to the solubility of substrates. High space–time-yields have been demonstrated to be correlated with high solubilities of substrates (Bommarius, 2001).

- *Enzymes require complicated co-substrates such as cofactors.* Much has been made of the requirement of some enzymes for cofactors, such as nicotinamide-containing compounds, NAD(P)(H), for dehydrogenases; flavin compounds, FMN or FAD, for oxidases; pyridoxylphosphate, PLP, for transaminases and decarboxylases; thiamine pyrophosphate, TPP, for carboligases, and vitamin B12 for glycerate dehydratase, among others. The scale-up of L-aspartate decarboxylation to L-alanine with the help of PLP-requiring L-aspartate decarboxylase, or of reductive amination of trimethylpyruvate to L-tert-leucine with the help of NADH-requiring leucine dehydrogenase demonstrates the feasibility of industrial processing with cofactor-requiring enzymes. The implementation also gives credence to the suggestion that cofactors are no longer the dominating cost component, as was believed until recently. Requirements for cofactors constitute a technological challenge but one that has been met successfully and so should not be regarded as impeding the use of biocatalysts in processing.

1.1.2 Current Advantages and Drawbacks of Biocatalysis

1.1.2.1 Advantages of Biocatalysts

The biggest advantage of enzymes is their often unsurpassed selectivity. While enzymes are used beneficially to increase chemical selectivity or regioselectivity of a reaction, their biggest advantage lies in the differentiation between enantiomeric substrates, a pair of substrates with Gibbs free enthalpy differences between the *R*- and the *S*-enantiomer ΔG_{RS} of around 1–3 kJ mol^{-1}. With enzymes, enantioselectivities of > 99% e.e. can be achieved routinely, although by no means in every case. This fact becomes increasingly important for using biocatalysts in the synthesis of advanced pharmaceutical intermediates, as regulatory agencies require separate toxicological studies for every impurity comprising above 1% of the content (Chapter 13, Section 13.1.4) (Crossley, 1995).

The fact that enzymes are active mostly at mild, near-ambient conditions of temperature and pH and preferentially in aqueous media is often regarded as an advantage rather than a drawback nowadays. Goals for industrial processing such as "sustainable development", "green chemistry", or "environmentally benign manufacturing", an increasingly important boundary condition for industrial activity in a large part of the world, would be much harder to attain without the availability of biocatalysts which tolerate and require such conditions.

Biocatalysts are able to catalyze an increasing breadth of reactions (see Table 1.4). This breadth translates into an increasing number of applications of biocatalysts on [and] industrial scale (Liese, 1999, 2000; Zaks, 2001; Straathof, 2002). Increasingly, biocatalysts are combined with chemical catalysts (Chapter 18) or utilized in a network of reactions in the cell ("metabolic engineering", Chapters 15 and 20).

1.1.2.2 **Drawbacks of Current Biocatalysts**

There are three essential drawbacks of today's biocatalysts:

1. biocatalysts are often not sufficiently stable in the desired media,
2. too few biocatalysts exist for the desired reactions from available substrates to targeted products, and
3. development cycles are too long for new and improved biocatalysts.

ad 1) *Biocatalysts are often not sufficiently stable in the desired media*. As mentioned above, this still is an essential drawback of biocatalysts. As even conformational changes of less than an Ångstrom can cause a precipitous decline in activity (Carter, 1988), retention of activity is a stringent criterion for the integrity of a protein molecule. Enzymes deactivate under a range of conditions such as extremes of temperature or pH value, physical forces such as cavitation by pumps and aqueous–organic or gas–liquid interfaces (Caussette, 1998), or specific covalent interactions (Quax, 1991; Kelly, 1994; Slusarczyk, 2000).

ad 2) *Too few biocatalysts exist for the desired reactions from available substrates to targeted products*. This argument can be approached from two vantage points. (We do not count the one that a higher demand than supply for biocatalysts speaks for itself.) As Table 1.4 (see below) reveals, there are biocatalysts for almost any reaction. However, most biocatalysts (there are now more than 4000 known) are either not well characterized, or proprietary, or at least not commercially available. The situation, though, improves steadily: just ten years ago, only about a dozen enzymes were available commercially, whereas nowadays the number has increased more than tenfold. Further rapid progress is to be expected.

ad 3) *Development cycles are too long for new and improved biocatalysts*. The development of some flagship biocatalytic processes of today took between 10 and 20 years: development of the acrylamide process took 20 years, of the Lonza process to L-carnithine 15 years. Compared to patent lifetimes around the same length, such durations can easily be deemed far too long for many applications. One reason for such timelines is the as-yet incomplete knowledge base of biotechnology and biocatalysis. With an improved knowledge base stemming from intensive research efforts, development times will certainly be decreased. Shortening the development cycle time for biocatalyts should therefore be a topic of active research. It is interesting, however, to pose the question whether long development times are particular for biocatalysis. Even a cursory inspection of the situation of homogeneous chiral chemical catalysis reveals that the situation is no different than for

biocatalysis. While the work of some outstanding researchers such as Ryori Noyori (Nagoya University, Nagoya, Japan), K. Barry Sharpless (Scripps Institute, La Jolla, CA, USA), who both shared the Nobel Prize for 2001, or Eric Jacobsen (Harvard University, Cambridge, MA, USA) has been scaled up and used in industrial processes, widely applicable design rules cannot be laid down yet for homogeneous chiral chemical catalysts.

1.2 Characteristics of Biocatalysis as a Technology

1.2.1 Contributing Disciplines and Areas of Application

From different disciplines, biotechnology and biocatalysis are seen from very different angles and perspectives (Figure 1.1). Chemistry and chemists emphasize a *molecularly-oriented* perspective dominated by compounds and transformations, whereas chemical engineering and thus chemical engineers favor a *process-oriented* perspective of reactions and processes; lastly, biology and its practitioners contribute a *systems-oriented* perspective of description at the organism level as well as in their view of evolution.

Different parts of each of the three disciplines are needed for the successful practice of biocatalysis: biochemistry and organic chemistry from chemistry; molecular biology, enzymology, and protein (bio)chemistry from biology; and catalysis, transport phenomena, and reaction engineering from chemical engineering are indispensable. Both biotechnology and biocatalysis are interdisciplinary areas; as most practitioners tend to hail from one of the three major contributing disciplines, hardly anybody has an equally strong command of all the sub-disciplines of biocatalysis.

There are not only many contributing disciplines for biotechnology and biocatalysis, but also many *areas of application:*

- production and transformation of compounds, mainly in the chemical and pharmaceutical industry,
- analytics and diagnostics, mainly in medicine, and
- environmental protection and bioremediation (reconstruction of the environment).

The areas of application differ from the *industries* which apply them; the most important ones are the pharmaceuticals, food, fine chemicals, basic chemicals, pulp and paper, agriculture, medicine, energy production, and mining industries (Figure 1.1).

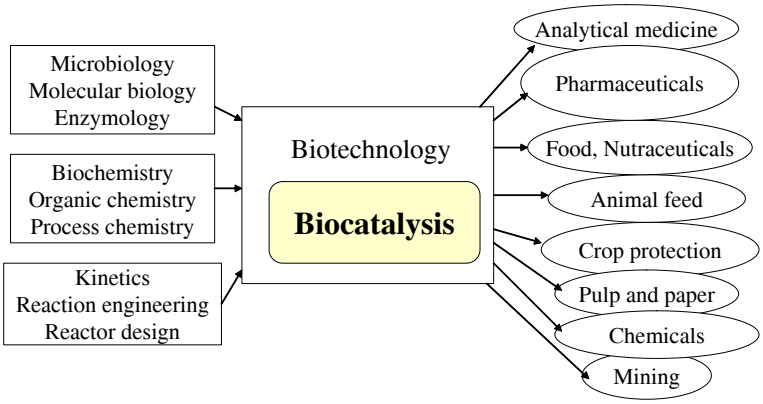

Figure 1.1 Central role of biocatalysis and biotechnology between interdisciplinary feeder sciences (biology, chemistry, chemical engineering science) and multiple user industries.

1.2.2
Characteristics of Biocatalytic Transformations

Biotechnological transformations include a broad range of processes, ordered according to the number of biologically performed process steps and the complexity of the substrates (Figure 1.2):

- *Fermentations* transform raw materials such as sugar, starch or methanol, often in industrial mixtures such as molasses or corn sleep liquor as carbon sources with living cells to more complex target products.
- *Precursor fermentations* start with defined educts and transform these, again with living cells, to the desired target products.
- *Biotransformations* transform defined precursors in a series of defined (not always known) steps with enzymes or resting cells to a desired target product.
- In *enzyme catalysis,* frequently *crude extracts* or *partially purified enzymes,* which only have to be free of side activities, are utilized for the transformation from defined substrate to target product.
- Purified enzymes are rarely used for the production of chemicals, possibly only for the production of highly priced fine chemicals such as pharmaceutical actives.

The limits between the areas are blurred: biotransformations and enzyme catalyses with crude extracts or pure enzymes are often summarized under the term "biocatalysis". "Biocatalytic processes" are taken to mean transformations of a defined substrate to a defined target product with one or several enzyme-catalyzed steps.

Biotechnology and biocatalysis not only differ from conventional processes by featuring a different type of catalyst but they constitute a new technology base:

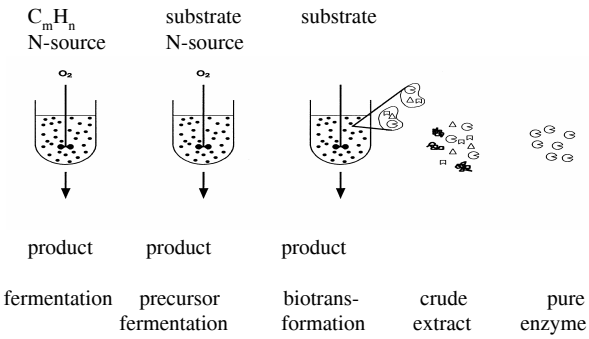

Figure 1.2 Biocatalysis as a continuum between fermentation and transformations with pure enzymes.

- Whereas the *raw materials base* of a conventional process is built on coal or oil, the base for biotechnology is sugar, lignin, or animal or plant wastes.
- Whereas conventional processes are dominated by unit operations such as distillation, often at high pressure or temperature, in biotechnology unit operations, such as membrane processes, chromatography or biocatalysis are prevalent.
- Whereas conventional *products* are often achiral organic molecules or polymers, the product range of biotechnological processes often encompasses chiral molecules or biopolymers such as proteins, nucleic acids, or carbohydrates.

1.2.2.1 Comparison of Biocatalysis with other Kinds of Catalysis

Compared with other kinds of catalysts, for example *homogeneous catalysts,* in which ligands are responsible for specificity, and *heterogeneous catalysts,* in which catalytically active centers are attached to solid carriers such as zeolites or metal oxides, enzymes feature the advantages and disadvantages listed in Table 1.1.

Whereas enzymes often feature great advantages in terms of selectivity, their stability is often insufficient. Additionally, long development times of new biocatalysts owing to an insufficient knowledge base of biocatalysis and biotechnology remain a problem and a challenge.

Table 1.1 Advantages and disadvantages of biocatalysts and enzymes.

Advantages	***Disadvantages***
Very high enantioselectivity	often low specific activity
Very high regioselectivity	instability at extremes of *T* and pH
Transformation under mild conditions	availability for selected reactions only
Solvent often water	long development times for new enzymes

1.2.3 Applications of Biocatalysis in Industry

1.2.3.1 Chemical Industry of the Future: Environmentally Benign Manufacturing, Green Chemistry, Sustainable Development in the Future

Owing to two very strong and important driving forces the chemical industry of the future will look considerably different from today's version:

- cost and margin pressure resulting from competition in an increasingly open market-oriented economy, and
- operation of the industry in a societal framework which puts emphasis on a clean (or at least less polluted) environment.

Processing with a view towards this new set of conditions focuses on the development of production routes with fewer processing steps, with higher yields on each step, to save material and energy costs. Less waste is generated, and treatment and disposal costs go down. Both pressures come together in the cases of environmental compliance costs.

In many cases, such as high-fructose corn syrup, or biotechnology and biocatalysis offer technology options and solutions that are not available through any other technology; in such situations such as acrylamide, nicotinamide or intermediates for antibiotics, biotechnology and biocatalysis act as "enabling technologies". In the remaining situations, biotechnology and biocatalysis offer one solution among several others, which all have to be evaluated according to criteria developed in Chapter 2: yield to product, selectivity, productivity, (bio)catalyst stability, and space–time-yield.

In this context, the three terms in the title are to a good extent synonymous; nevertheless, they have been developed in a slightly different context:

- *environmentally benign manufacturing* is a movement towards manufacturing systems that are both economically and environmentally sound;
- *sustainable development* is a worldwide Chemical Industry movement and represents a set of guidelines on how to manage resources such that non-renewables are minimized as much as possible;
- *green chemistry* is the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances.

"Green chemistry is an overarching approach that is applicable to all aspects of chemistry" (Anastas, 2002). Green chemistry methodologies can be viewed through the framework of the "Twelve Principles of Green Chemistry" (Anastas, 1998):

1. It is better to prevent waste than to treat or clean up waste after it is formed.
2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity towards human health and the environment.

4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.
5. The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible, and should be innocuous when used.
6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperatures and pressures.
7. A raw material or feedstock should be renewable rather than depleting wherever technically and economically practicable.
8. Unnecessary derivatization (blocking group, protection/deprotection, temporary modification of physical/chemical processes) should be avoided wherever possible.
9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. Chemical products should be designed so that at the end of their function they do not persist in the environment and they do break down into innocuous degradation products.
11. Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. Substances and the form of a substance used in a chemical process should be selected so as to minimize the potential for chemical accidents, including releases, explosions, and fires.

Catalysis offers numerous advantages for achieving green chemistry goals: novel, high-yield, shorter process routes; increased selectivity; and lower temperatures and pressures. Biocatalysis combines the goals of all three topics above. Biocatalysts, as well as many of the raw materials, especially those for fermentations, are themselves completely renewable and for the most part do not pose any harm to humans or animals. Through the avoidance of high temperatures and pressures and of large consumptions of metals and organic solvents, the generation of waste per unit of product is drastically reduced.

1.2.3.2 Enantiomerically Pure Drugs or Advanced Pharmaceutical Intermediates (APIs)

The most important property of a catalyst for application in a process is not its activity but rather its selectivity, followed by its stability, which is just activity integrated over time. In comparison with other catalysts, enzymes often feature superior selectivity, especially regio- and enantioselectivity. Enzymes are destined for selective synthesis of molecules with several similar functional groups or chiral centers. A growing emphasis is laid on the synthesis of enantiomerically pure compounds (EPCs). The interest in EPCs shown by all areas of the life science industries (e.g., pharmaceuticals, food and agriculture), stems from the challenge to develop structurally optimized inhibitors, almost always containing chiral centers.

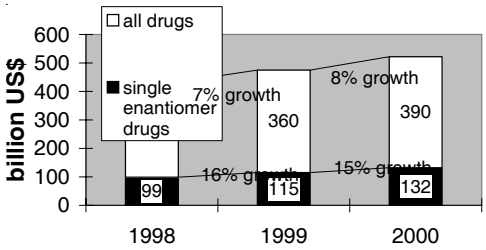

Figure 1.3 Relevance of chiral drugs (May, 2002).

The importance of the drive for enantiomeric purity for biocatalysis can be discerned from the fact that fully 88% of all biocatalytic processes above a scale of 100 kg involve chiral targets (Straathof, 2002). Of the new drugs introduced in 2000, 76% were single enantiomers compared with 21% in 1991 (Agranat, 2002). As Figure 1.3 reveals, the revenues from single-enantiomer drugs have exceeded $ (or €) 100 billion in 2000, compared to $ (€) 500 billion total, and feature a higher growth rate than non-single-enantiomer drugs (155 vs. 8% annual growth) (May, 2002).

The whole of Chapter 13 is devoted to the application of biocatalysis in pharmaceutical syntheses.

1.3 Current Penetration of Biocatalysis

1.3.1 The Past: Historical Digest of Enzyme Catalysis

Table 1.2 provides an overview of historic events in biocatalysis and biotechnology. It demonstrates that biotechnology is an old science, or even an old art. The big ideas and driving forces for biocatalysis in the 20th century were twofold: first, the idea of catalysis as transition-state complementarity in 1944 and second, the development of molecular biology after 1978 to allow the design of enzymes and their production vehicles.

1.3.2 The Present: Status of Biocatalytic Processes

Even a few years ago, biocatalytic processes on an industrial scale were few. As with so many novel technologies, however, the time lag between research, development, and large-scale application just had to pass before we could witness a range of such processes in industrial practice today. In a review summarizing the status of biocatalytic processing in industry, Straathof (2002) records that the number of industrial-scale biocatalytic processes has more than doubled over the last 10 years.

Straathof included 134 industrial-scale biotransformations, counting processes on a scale of > 100 kg with whole cells or enzymes starting from a precursor other than a C-source. Among the highlights of this review were the facts that:

Table 1.2 Historical digest of enzyme catalysis and biotechnology.

Year(s)	*Who?*	*Where?*	*What?*
BC	Unknown	Old World	Chymosin from calf and sheep stomach utilized for production of cheese
1783–1836	Spallanzani		verifies in vitro "digestion" of meat in stomach juice: factor called "pepsin"
1876	Kühne		term "enzyme" for catalysts not bound to living cells ("unorganized ferments")
1877	Eduard Buchner (Nobel prize 1907)	Berlin Agricultural College, Germany	1st alcoholic respiration with cell-free extract: vital force, *vis vitalis*, does not exist
1893	Wilhelm Ostwald	Leipzig Univ., Germany	definition of term "catalyst" (Nobel prize 1909)
1894	Emil Fischer	Berlin Univ., Germany	"lock-and-key" concept (Nobel prize 1902)
1903	Henry D. Dakin	London, UK	1st enantioselective synthesis, with oxynitrilase
1908	Otto Röhm	Darmstadt, Germany	Patent for enzymatic treatment of leather (with trypsin)
1913–1915	Röhm Company	Darmstadt, Germany	1st laundry detergent with enzyme (pancreatin): "Burnus"
1926	James B. Sumner	Cornell Univ., Ithaca, NY, USA	1st enzyme crystallized: urease from jack beans (Nobel prize 1946)
1936	Ernst Sym		lipase reaction in organic solvent
1944	Linus Pauling	Caltech, Pasadena, CA, USA	1st attempt to explain catalysis as transition-state complementarity
1950	Pehr Edman	Univ. of Lund, Sweden	protein degradation developed
1951	Frederick Sanger and Hans Tuppy	Univ. of Cambridge, UK	sequence determination of insulin β-chain: each protein is characterized by a sequence (Nobel prize 1978)
1960		Novo (Bagsvaerd, Denmark)	large-scale protease production from *Bacillus licheniformis* in submerged culture
1963	Stanford Moore and William Stein	Rockefeller Univ., New York, USA	amino acid sequence of lysozyme and ribonuclease elucidated (Nobel prize 1972)
1978	Stanley Cohen and Herbert Boyer	Stanford, CA, USA	method of recombination of DNA developed
1985	Michael Smith	Univ. of British Columbia, Canada	site-directed gene mutagenesis to change enzyme sequence (Nobel prize 1993)
1988	Kary B. Mullis	Cetus Corp., CA, USA	invention of PCR (Nobel prize 1993)
2000	Celera Genomics	Gaithersburg, MD, USA	sequencing of human genome announced (3 billion basepairs)

Table 1.3 Biotransformations on an industrial scale.

Production scale [tpy]	*Product*	*Enzyme*	*Reactor*	*Company*
> 1 000 000	high-fructose corn syrup (HFCS)	glucose isomerase	fixed-bed, IME	various
> 100 000	lactose-free milk	lactase	fixed-bed, IME	various
> 10 000	acrylamide	nitrilase	batch reactor	Nitto Co.
	cocoa butter*	lipase (CRL)	fixed-bed, IME	Fuji Oil
> 1,000				
	nicotinamide	nitrilase	3-stage batch	Lonza Guangzhou
	D-pantothenic acid	aldonolactonase		Fuji Pharmaceuticals
	(S)-chloropropionic acid	lipase		Dow Chemical
	6-aminopenillanic acid	penicillin amidase	fixed-bed, IME	various
	7-aminocephalosporanic acid	glutaryl amidase	Kundl/Hoechst	
	aspartame®	thermolysin	soluble enzyme	Tosoh/DSM
	L-aspartate	aspartase	fixed-bed, IME	various
	D-phenylglycine	hydantoinase/ (carbamoylase)	resting cells	Kanegafuchi
	D-*p*-OH-phenylglycine	hydantoinase/ carbamoylase	resting cells	Recordati
> 100	ampicillin	penicillin amidase	stirred IME	DSM–Gist Brocades
	L-methionine, L-valine	aminoacylase	EMR	Degussa (Rexim)
	L-carnitine	dehydrase/ hydroxylase	whole cells	Lonza
	L-dopa	β-tyrosinase		Ajinomoto
	L-malic acid	fumarase	fixed-bed, IME	Tanabe
	(*S*)-methoxyisopropylamine	lipase	repeated batch	BASF
	(*R*)-HPOPS	hydroxylase	batch reactor	BASF
	(*R*)-mandelic acid	nitrilase	batch reactor	BASF
	L-alanine	L-aspartate-β-decarboxylase	IME	various

tpy: (metric) tons per year; IME: immobilized enzyme reactor; EMR: enzyme membrane reactor; L-dopa: 3,4-dihydroxyphenylalanine; (*R*)-HPOPS: (*R*)-2-(4-hydroxyphenoxy)propionic acid.

* Operation depends on the price of substrate palm oil vis-à-vis competing sources.

- hydrolases (44%), followed by oxidoreductases (30%), dominate industrial biocatalytic applications, and
- average performance data for fine chemicals (not pharmaceuticals) applications are 78% yield, a final product concentration of 108 g L^{-1}, and a volumetric productivity of 372 g $(L \cdot d)^{-1}$.

Performance data for biocatalytic reactions and processes will be discussed throughout this book, especially in Chapters 2, 7, 18, and 19. Table 1.3 lists some of the largest-scale biocatalytic processes, categorized by size stated as of tons per year (tpy).

1.4 The Breadth of Biocatalysis

1.4.1 Nomenclature of Enzymes

According to the first report of the Enzyme Commission from 1961, all enzymes are classified in six enzyme classes, depending on the reaction being catalyzed. Within the scheme of identification , each enzyme has a number ("E.C." stands for "Enzyme Commission"):

E.C. a.b.c.d

a: enzyme class (of six) **b**: enzyme-subclass
c: enzyme sub-subclass **d**: enzyme sub-sub-subclass (running number)

The number of known enzymes has risen significantly, from 712 in the first edition of *Enzyme Nomenclature* of 1961 through 2477 in 1984 to 3196 in 1992, the year of the third edition. It is important to note that this classification scheme does not organize enzymes according to amino acid sequence or type of three-dimensional structure, and in principle not even according to chemical mechanism.

1.4.2 Biocatalysis and Organic Chemistry, or "Do we Need to Forget our Organic Chemistry?"

The question is often posed of whether biocatalysts operate by different scientific principles from organic catalysts. Careful analysis reveals that they do not (Knowles, 1991; Menger, 1993). Enzymes are "not different, just better" (Knowles, 1991). The multitude of enzyme models including oligopeptidic or polypeptidic catalysts (Chapter 18) proves that all enzyme action can be explained by rational chemical and physical principles. However, enzymes can create unusual and superior reaction conditions, such as extremely low pK_a values for a lysine residue (Westheimer, 1995) or a high positive potential for a redox metal ion (Wittung-Stafshede, 1998).

Enzymes increasingly have been found to catalyze almost any reaction of organic chemistry. Table 1.4 provides examples for a series of reactions.

The principles of how and why enzymes work are discussed in the next chapter.

Table 1.4 Chemical reactions and their chemical and enzymatic manifestations.

Reaction	*E.C. number*	*Enzyme*	*Reference*
Meerwein–Ponndorff–Verley reduction	1.1.1.1	alcohol dehydrogenase	
Oppenauer oxidation	1.1.1.1	alcohol dehydrogenase	
Baeyer–Villiger oxidation	1.14.13.22	BV monooxidase (CHMO)	Taschner, 1988
Ether cleavage	1.14.16.5	glyceryl etherase	Kaufmann, 1997
Disproportionation	1.15.1.1	superoxide dismutase	McCord, 1969
Etherification	2.1.1.6	COMT	Mannisto, 1998
Transamination	2.6.1.x	aminotransaminase	Stirling, 1992
Hydrolysis Phosphate hydrolysis	3.1.–.– 3.1.3.2/1	lipase, esterase phosphatase, acid/alkali	
Esterification	3.4.21.14	subtilisin	
Transesterification	3.4.21.14	subtilisin	
Oximolysis	3.1.1.3	lipase	Gotor, 1990
Electrophilic addition	4.2.1.2	fumarase	
Aldol reaction	4.1.2.x	aldolase	Wymer, 2001
Decarboxylation (β-elimination)	4.1.1.12	L-asp. decarboxylase	Chibata, 1984
Mannich reaction	4.1.99.1 4.1.99.2	tryptophanase β-tyrosinase	
Diels–Alder reaction		Diels–Alderase	Oikawa, 1995
Amadori rearrangement	4.1.3.27	PRAI	Jürgens, 2000
Claisen rearrangement	5.4.99.5	chorismate mutase	Pawlak, 1989
Racemization	5.1.2.2	mandelate racemase	Kenyon, 1995
Isomerization	5.3.1.5	glucose isomerase	
Kolbe–Schmitt reaction	6.4.–.–	phenol carboxylase	Aresta, 1998
Ligation	6.5.1.1	DNA ligase	

BV monooxygenase: Baeyer–Villiger monooxygenase; CHMO: cyclohexanone monooxygenase; COMT: catechol *O*-methyltransferase; PDC: pyruvate decarboxylase; PRAI: phosphoribosyl anthranilate isomerase.

Suggested Further Reading

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