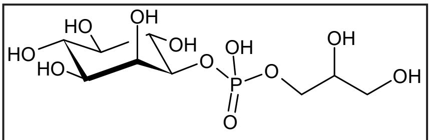
Chapter 1

Introduction

The statement that biocatalysis is of great importance sounds trivial in face of the fact that biocatalysis is the prerequisite for any life at all. Application of biocatalysis has a more than eight thousand year old tradition if the unwitting employment of the underlying processes in ancient times is included, e.g. fermentation in connection with beer brewing or the baking of bread. Nowadays research in biocatalysis increasingly influences all areas of daily life, including medicine, pharmacy, nutritional products, analytics, environmental technology, and others.

Millions of years of evolution have generated an unimaginable diversity of organisms. Biocatalysts regulate and control all metabolic reactions in microorganisms, plants and animals in an often very selective way and under conditions that are either mild or adapted to the special requirements of the milieu an organism develops its activities in. For instance, microorganisms have been found to live under unusual environmental conditions such as disused chemical plants by developing an enzyme equipment enabling them to grow on the chemicals they discover. Among the so-called extremophiles (Berger *et al.*, 2014, and literature cited therein) barophiles populate deep sea habitats where they grow at temperatures just above 0°C, and at a pressure of more than 1000 bar but perish when temperature increases and the pressure decreases; psychrophiles metabolize at even lower temperatures, e.g. under the conditions of the Siberian permafrost. Of even more biotechnological relevance are thermophilic (moderate-, extreme-, and hyperthermophilic) microorganisms existing in so-called ecological niches, as found, e.g. in the hot springs of the Yellow Stone National Park about 60 years ago. They produce enzymes that function at temperatures up to 130°C and often under extreme pH-conditions (alkalophiles or acidophiles). Such robust enzymes are of great interest for various biotechnological processes. Interestingly, not all enzymes isolated from (hyper)thermophiles are particularly heat stable. These organisms developed alternative strategies to survive under such conditions as, e.g. the synthesis of low-molecular mass metabolites that exert *in vivo* a stabilizing effect resulting from strengthening intramolecular interactions within the protein molecule with a stabilizing effect on protein unfolding as shown by Roychoudhury *et al.* (2013) by means of atomic force microscopy. To these so-called compatible solutes belong the disaccharide trehalose, α- and β-glutamate (accumulated for osmoadaption), di-*myo*-inositol phosphate and 1-glyceryl-1-*myo*-inositol phosphate (opposite figure), discovered in the hyperthermophilic bacterium *Aquifex pyrophilus* in response to both osmotic and heat stresses (Lamosa *et al.*, 2006). These solutes are of interest for a variety of biotechnological applications. Altogether, there should exist innumerable different enzymes (most of them not detected so far) to catalyze nearly all types of chemical reactions known from Organic Chemistry lessons. The application potential of biocatalysts for chemical synthesis has been recently reviewed by Clouthier and Pelletier (2012), and is discussed here in a variety of chapters, particularly in connection with the topic Industrial Biocatalysis.



Phosphoric acid 2,3-dihydroxy-propyl ester 2,3,4,5,6-pentahydroxy-cyclohexyl ester

1.1 Advantages and Disadvantages of Biocatalysts

Nature has created excellent catalysts by evolution over millions of years. They are mostly proteins (enzymes or catalytic antibodies) but also nucleic acids with catalytic properties similar to those of enzymes detected in the early 80s. Up to now, of these naturally occurring catalysts only enzymes are used in applied biocatalysis.

Enzymes catalyze chemical reactions (and energetic transformations) in a single cell or in a whole organism, essential for survival and reproduction. A biocatalyst may either be the complete cell itself, employed in a viable, non-viable, growing or non-growing state, or an individual enzyme. As other catalyst, they increase the rate at which equilibrium is attained without affecting the equilibrium constant by providing an alternative reaction path with lower activation energy than the one of the corresponding un-catalyzed reaction (Chapter 5). What is spectacular is the degree of rate acceleration.

Table 1.1 The main advantages and disadvantages of biocatalysts with regard to their possible application in biotransformations on laboratory or industrial scale.

Advantages	Disadvantages
▪ Very efficient catalysis of most known chemical reactions ▪ High regio- and stereoselectivity ▪ Mild reaction conditions and thus low energy consumption ▪ Amount of byproducts is low ▪ They are biodegradable ▪ Preparation on large scale is possible through fermentation (microbial enzymes) ▪ Reuse is possible (immobilization) ▪ They can be designed to a certain extent ▪ They are non-toxic if correctly applied	▪ Protein molecules are rather instable in aqueous media ▪ Many enzymes are cofactor-dependent ▪ Allergic reactions are possible Enzymes may be inactivated by ▪ higher temperatures ▪ extreme pH-values ▪ higher salt concentrations ▪ (polar) organic solvents Inactivation may further occur through inhibition by ▪ substrate ▪ product ▪ metal ions ▪ inhibitors

An impressive example is given by the enzyme catalase that catalyzes the decomposition of hydrogen peroxide. From the activation energies for the uncatalyzed and the catalyzed reaction, i.e. 75 kJ/mol and 8 kJ/mol, respectively, results a factor of rate enhancement of about 10^{15}. This individual value lies at the upper limit; usually such factors for enzyme-catalyzed reactions are between 10^8 and 10^{12}.

The enormous rate acceleration allows reactions to proceed under physiological conditions in a split second that would take ages to reach equilibrium without a catalyst. Enzyme-catalyzed reactions are often highly substrate-specific without forming byproducts, a consequence of their regiospecificity. Furthermore, due to the fact that enzymes are asymmetric molecules, they may precisely differentiate between stereoisomers, resulting in the formation of chiral products whereas chemical synthesis often leads to racemic mixtures. All these properties make enzymes interesting candidates as catalysts for industrial processes. However, enzymes also have disadvantages (Table 1.1) limiting their use.

1.2 Strategies to Improve the Performance of Biocatalysts

Not long ago enzymes had to be employed as provided by Nature. However, with the advent of new biological and molecular tools it became possible to influence the properties of biocatalysts with respect to catalytic activity, selectivity, and stability. High stability of a biocatalyst under process conditions is a prerequisite for its economic application in the industrial production of high-value fine chemicals as well as bulk compounds, in order to create a competitive alternative to traditional chemical procedures. This does not necessarily mean that an existing chemical process will always be entirely substituted by an application of biotechnological methods, but it is to be expected that they will be combined to an increasing extent with conventional chemical technologies, thus contributing to a reduced use of hazardous substances, to minimize energy consumption, and to a reduction in waste generation. Another important aspect is to integrate, wherever possible, renewable raw materials in production processes; for reviews see e.g. Zechendorf, 1999, Busch *et al.*, 2006; Soetaert and Vandamme, 2006.

A variety of biotechnological tools have been developed in the last two decades to make enzyme- or whole-cell-catalyzed reactions more efficient under given process conditions (Bornscheuer *et al.*, 2012; see also Chapters 15, 16). The metabolic engineering of production strains such as the often employed *Escherichia coli* by insertion of a foreign gene into its genome or by transformation with a plasmid containing the desired gene has benefited from recent developments in what is known as systems biology enabling amongst others a holistic view on biocatalysis. This approach is based on one of the famous statements made by the Greek philosopher Aristotle (384 to 322 BCE; a pupil of Plato and a teacher of Alexander the Great): “The whole is more than the sum of its parts” (shortened version). Hence, as, e.g., discussed by

Kitano (2002) in his brief overview on systems biology, a comprehensive understanding of biology requires an insight into structure and dynamics of cellular and organismal function. This means that the properties of a biological system cannot be adequately described by an isolated look at genes and/or proteins being present but has to consider gene interactions and metabolic pathways, the influence of parameters varying with time, mechanisms involved in the control of a cell's state, as well as questions concerning the design of systems with the aim to change their properties. This has led to several rather new subdisciplines of modern biology dealing with the entirety (characterized by the suffix –ome) of, e.g., the genetic information (genome), mRNA species (transcriptome), proteins (proteome), *etc*., analyzed by the respective omics techniques such as genomics, transcriptomics, and proteomics, respectively. These analyses generate a huge amount of data processed by means of bioinformatics software developed to generate new biological information.

1.3 Biocatalysis — An Interdisciplinary Science

Biocatalysis is one of the main pillars of applied biotechnology, defined by the European Federation of Biotechnology as the "*integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogs for products and services*"; according to EuropaBio, 2003, "*White Biotechnology is the application of Nature's toolset to industrial production*" (Chapter 20) Both definitions have in common that biotechnology, and thus biocatalysis, are looked at as interdisciplinary sciences. Application of biotechnology requires basic knowledge in the areas shown in Fig. 1.1.

The different subjects related to biotechnology are more traditional ones (biology, chemistry, physics, mathematics), and comparatively new ones as bioinformatics and material science. Research in biotechnology implies that those who are active in this field, though being specialized, e.g., in enzymology should have at least a fundamental knowledge about the neighboring disciplines as a prerequisite for facilitating cooperation. At the same time this interdisciplinarity mirrors the complexity of many scientific problems and has to be considered when teaching natural sciences (Section 1.4).

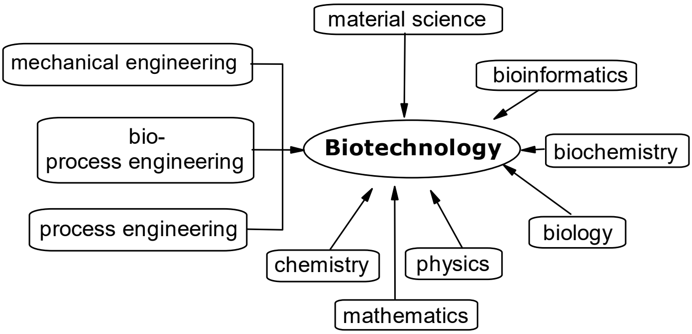


Fig. 1.1 Some of the scientific disciplines contributing to biotechnology; they are of relevance for biocatalysis, too, and comprise the traditional subjects (chemistry, physics, *etc.*) as well as several new ones as e.g. 'bioinformatics' and 'material science'.

Interdisciplinarity in this field becomes apparent by designing a process involving a biocatalyst (enzyme or whole cells). The search for the desired catalyst requires a screening based on more or less sophisticated analytical methods. If this has been successful, it has to be clarified whether its properties (kinetic behavior, stability, *etc.*) match the respective process conditions. A whole arsenal of methods is available for the improvement of these properties. The biocatalyst may be, e.g., immobilized by various different procedures to enhance its operational stability. Temperature stability, solvent tolerance, *etc.* of an enzyme may be improved by rational design, requiring knowledge about the structure obtained from NMR-spectroscopy and/or X-ray diffraction; alternatively, there are evolutionary methods, or the use of the catalyst in the shape of designer bugs based on metabolic engineering. Additional aspects ranging from possible cofactor regeneration to environmental measures must be considered to design a process, capable of competing with existing traditional ones as illustrated by Schmid *et al.* (2001) by the biocatalysis cycle. Meanwhile well-established processes are the production of high-fructose syrup with xylose isomerase (isomerization of glucose to fructose), the use of penicillin amidase for the synthesis of semisynthetic penicillins, or the employment of nitrile hydratases for the conversion of acrylonitrile to acrylamide by hydration of the substrate. These examples together with many others are discussed in Chapter 10.

1.4 The Impact of Biocatalysis on Teaching Natural Science

Emil Fischer's famous statement about the links between biology and chemistry made more than a century ago (Fischer, 1907) is as relevant today as it ever was. In his Faraday Lecture to the Chemical Society about Synthetic Chemistry and its Relation to Biochemistry he said "*…the separation of biology and chemistry was necessary while experimental methods and theories were being developed. Now that our science is provided with a powerful armoury of analytical and synthetic weapons, chemistry can once again renew the alliance with biology, not only for the advantage of biology but also for the glory of chemistry*." However, unlike at the beginning of the 20th century the development of (natural) sciences affects our society to an increasing degree in connection with social aspects as well as decision making processes. A topical example is biofuel production, based on the idea that the amount of CO_2 emitted by its combustion is equal to that absorbed during growth of the crop. The biofuel boom, however, has great drawbacks. Forests, known as CO_2 sinks are destroyed in favor of extensive farming. Maize (bioethanol) and rapeseed (biodiesel) as profitable sources for biofuel require large amounts of nitrogen fertilizers with the consequence of a green-house gas effect by extra N_2O emission estimated to be up to 70% higher than resulting from fossil fuel (Crutzen *et al.*, 2007). And worst of all, people in developing countries suffer from this biofuel euphoria in that prizes for staple foods increased dramatically in recent years; thus, apart from starvation, increasingly less money is left for health care and education. Hence there is now a move towards advanced — 2nd generation — biofuels (Chapter 20) produced from agricultural waste, non-food bio-mass, microalgae, *etc.* (Carriquiry *et al.*, 2010).

If there is a principle agreement that every future citizen should understand the basic economical and ethical issues of natural sciences it is self-evident that the corresponding school as well as university curricula have to be adapted to the development in natural sciences. In this connection, different problems — apart from a certain still existing conservatism of some of those responsible for revising, e.g., chemistry curricula — exist; two main problems are the enormously increasing knowledge within ever shorter time intervals together with the fact that the time available for teaching remains constant (or even decreases). From this the question arises which contents of the existing curricula should be omitted and what would have to be added. A far-reaching proposal for chemistry curricula has been made by David Samuel some 30 years ago in a lecture given at the 29th IUPAC congress in 1983. David Samuel recommended the content of chemistry curricula to be geared to Life Sciences; some examples of topics that should be treated are phosphorous chemistry, lipids and lipid membranes, the structure and properties of macromolecules, the chemistry of glycoproteins, *etc.*, but also the basic concepts of Physical Chemistry.

A similar way to overcome some of the problems chemistry teachers (at school as well as university) today are faced with is to orientate the curriculum content, at least in part, on the chemistry of everyday life. This not only motivates students to occupy themselves with Natural Sciences but also allows the treatment of actual topics with simultaneously introducing students to the fundamentals of chemistry related to that topic. A good example is again ‘Biocatalysis’, traditionally taught in biochemistry lessons and corresponding lab courses. Biocatalysis, however, is also well-suited for treating catalysis in connection with kinetics in a practical course in Physical Chemistry. If this is done, e.g., with the urease-catalyzed urea hydrolysis, conductivity measurements can be used to determine the reaction rate (Hanss and Rey, 1971a, b; Lawrence *et al.*, 1972), thereby offering at the same time the possibility to teach in parallel fundamentals concerning the behavior of electrolytes — a time-saving teaching method. Urease may be also immobilized by easy-to-perform preparative chemistry, and a discussion of results obtained from experiments with immobilized urease in comparison with those resulting from respective investigations into the behavior of the native enzyme inevitably leads to the laws of heterogeneous catalysis. Furthermore, the design of immobilized biocatalysts with regard to optimized properties concerning, e.g., storage and operational stability provides a link to Materials Sciences

(Grunwald, 2006; 2013). A particular advantage of this concept of linking topics of General Chemistry to aspects of Biochemistry and especially Biocatalysis (and *vice versa*) is that corresponding curricula reflect the progress in research and thereby help to keep teaching up-to-date.

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