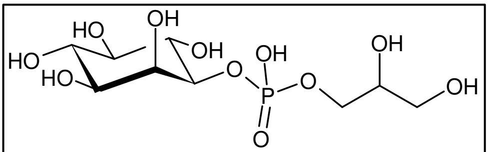
Chapter 1

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 long 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. In present days, the emerging research results in life science, and especially in biocatalysis, increasingly influence all areas of daily life. Modern developments in medicine, pharmacy, nutritional products, analytics, environmental technology, and others are inconceivable without the innovative research results in biocatalysis.

Millions of years of evolution have generated an unimaginable multiplicity of organisms. Biocatalysts regulate and control all metabolic reactions in microorganisms, plants and animals in a very selective way and make the necessary high reaction rates possible. Reaction conditions are either mild or adapted to the special requirements of the milieu an organism develops its activities in. Microorganisms have been found to live under unusual environmental conditions, e.g. disused chemical plants; they adapted to their surroundings by developing an enzyme equipment that enables them to degrade the chemicals they discovered. So-called 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 extremophiles – microorganisms existing in so-called ecological niches, first found in the hot springs of the Yellow Stone

National Park about 50 years ago. They produce enzymes that function at temperatures up to 130°C and extreme pH-values. Such robust enzymes are of great interest for various biotechnological processes. Interestingly, not all enzymes isolated from hyperthermophiles are particularly heat stable. These organisms obviously developed alternative strategies to survive under such conditions as e.g. to synthesize low-molecular mass metabolites exerting *in vivo* a stabilizing effect *via* an interaction with proteins by a mechanism, not fully understood so far. To these so-called compatible solutes belong α- and β-glutamate (accumulated for osmoadaption), di-*myo*-inositol phosphate and 1-glyceryl-1-*myo*-inositol phosphate (opposite figure), recently discovered in the hyperthermophilic bacterium *Aquifex pyrophilus* in response to both osmotic and heat stresses (Lamosa *et al*., 2006). The interesting question whether these solutes can be used to stabilize other isolated enzymes still needs to be elucidated. Altogether, there should exist innumerable different enzymes (most of them not detected so far) to catalyse most types of chemical reactions. In view of these facts the importance of biocatalysis lies in its enormous potential for application becoming more and more evident in recent years as new sensational research results emerge.



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

Not long ago enzymes had to be employed as provided by Nature. However, as new biological and molecular tools as mutagenesis or recombinant technologies became available, it was 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.

Parallel to the development of knowledge in this field, new terms as *proteomics*, *genomics designer bugs*, *etc.* have emerged, some of which were not to be found in relevant textbooks or dictionaries 10 or 15 years ago. Structural genomics stand for all methods by which highly resolved structural information of all proteins coded by a genom are gained. The aim of proteomics is to identify and characterize all proteins within e.g. a cell, together with an analysis of their interactions under varying conditions, making proteomics rather complex. With these and other methods and strategies metabolic pathways of organisms can be elucidated in detail; corresponding results establish to an increasing degree the basis for the optimisation of bioprocesses or for a targeted change of the catalytic profile of a microorganism by genetic modification.

Nowadays, research in natural sciences is shaped considerably by results in life sciences *and* material sciences, with nanosciences taking a prominent role in the latter field. So-called smart materials have been found in recent years, responding to parameters of their surroundings, i.e. temperature, pH-value, or salt concentrations. They have been successfully employed in the controlled release of pharmaceutically active compounds as well as in the field of immobilized biocatalysts. The immobilization of enzymes onto the surface of nano-structured materials with the aim to produce a biosensor is an example for ‘applied nanobiotechnology’. All these developments demonstrate the multidisciplinary character of the research in biocatalysis. Emil Fischer was probably first in formulating this in a visionary way already in 1907 in his Faraday Lecture to the Chemical Society about ‘Synthetic Chemistry’ and its Relation to Biology when he said: “*….the separation of chemistry from biology 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, it took approximately seven further decades until the cooperation between chemists and biologists (and scientists of other research fields) by and by became a matter of course.

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

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also nucleic acids with catalytic properties similar to those of enzymes detected in the early 80s. Up to now, of these natural 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. The properties of enzymes, compared with catalysts normally used in chemical processes, are remarkable. Enzymes exist for nearly all reactions known in Organic Chemistry. Characteristic for each catalyst, they increase the rate at which equilibrium is attained without effecting the equilibrium constant by providing an alternative reaction path with a lower activation energy than the one of the corresponding uncatalyzed reaction. What is spectacular is the degree of rate acceleration.

Table 1.1: Some of the main advantages and disadvantages of biocatalysts against the background of 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 They 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 ▪ Many enzymes are cofactor-dependent ▪ Allergic reactions possible

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^{10} and 10^{12}.

Apart from this, 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 have the ability to 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, as always, enzymes also have disadvantages limiting their use as catalysts in chemical reactions. Due to their flexible molecular structure they are rather instable in aqueous media, and are inactivated by higher reaction temperatures, or extreme pH values and salt concentrations. They may be inhibited by their own substrate, by the reaction product and/or by other compounds. Another disadvantage is that most enzymes considered for application in biocatalysis outside of the producing cell require cofactors or coenzymes. Finally, enzyme molecules, if inhaled as small particles in the lung, may cause allergic reactions when transferred directly into the blood stream; they are, however, non-toxic if correctly used.

However, the properties of biocatalysts listed in table 1.1 are not fixed but undergo a permanent change, the more so since the potential of evolutionary methods was discovered (chapter 12). It provides the possibility to not only to reduce production costs but also to improve their properties so that disadvantages as low heat stability, poor tolerance to organic solvents, *etc.*, may be considerably reduced. This is vital for making biotransformations competitive, compared to processes employing traditional (bio)catalysts, and thus is a prerequisite for what is called White (or Industrial) Biotechnology that makes use of the many advantages of biocatalysts to develop sustainable technologies for both the production of high added-value products, e.g. vitamins, as well as bulk chemicals, including polymers (chapters 16 and 17). This does not mean that an existing chemical process will always be entirely substituted by White Biotechnology, but biocatalysis will be combined to an increasing extent with conventional chemical technologies, thus contributing to a reduced use of hazardous and/or dangerous substances, to minimized energy consumption, and to a reduction in waste generation. Another important aspect is to integrate, wherever possible, renewable raw materials (mainly provided by agriculture and forestry) in the production process. The source of renewable raw materials is biomass, estimated to be produced to an amount of about 170 billion tons per year and consisting mainly of carbohydrates (75%) and lignin (20%). Due to their natural basis, biotechnological processes are more advantageous than chemical processes for the adaptation of renewable raw materials into starting materials for chemical synthesis where biocatalysis plays an increasing role; for reviews see e.g. Zechendorf, 1999, Busch *et al.*, 2006; Soetaert and Vandamme, 2006.

1.2 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*", and according to EuropaBio, 2003, "*White Biotechnology is the application of Nature's toolset to industrial production*". Both definitions have in common that biotechnology, and thus biocatalysis, are looked at as interdisciplinary sciences. Application of biotechnology requires knowledge (at least a basic one) 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. It is characteristic for modern science that the term 'biotechnology' in Fig. 1.1 may be replaced by other subjects, e,g, 'materials science' due to its importance for many areas including biotechnology, medicine *etc.*; furthermore, the key-technology *nanoscience* finds promising applications in areas as different as medicine, electronics, biotechnology and several others;

nanotechnology would not exist without competent chemists knowing how to prepare nanoparticles for a certain application which are then analyzed for their properties (structure, magnetic behavior *etc.*) by physicists.

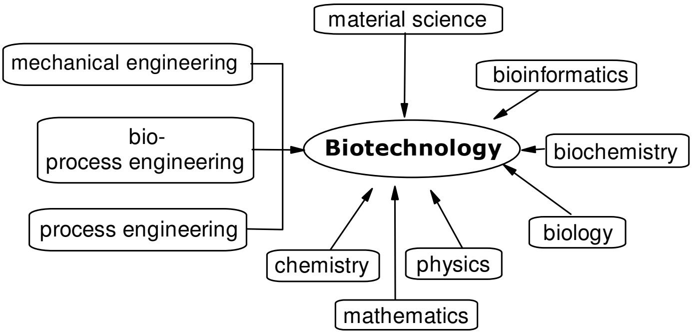

Fig. 1.1: Some of the scientific disciplines contributing to research and development in biotechnology; they are of relevance for biocatalysis, too, and comprise the traditional subjects (chemistry, physics, *etc*) as well as several new ones as e.g. ‘bioinformatics’ and ‘material science’.

Transferred to biotechnology, this 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 (chapter 1.3). 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. 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. 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 several others are discussed in chapter 10.

1.3 The Impact of Biocatalysis on Teaching Natural Science

Emil Fischer's statement about the links between biology and chemistry made more than a century ago (chapter 1.1) is as relevant today as it ever was. 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 greenhouse gas effect by extra N_2O emission estimated to be up to 70% higher than that 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.

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 more than 25 years ago in a lecture given at the 29th IUPAC congress in 1983. What would, e.g., not be missed though is still being kept in many curricula for the first years at university is all the out-dated wet chemistry that was important for qualitative and quantitative Inorganic Chemistry before the powerful physical methods available today were known. With respect to Organic Chemistry one should discuss whether it is really necessary for students to learn how compounds are prepared by elaborate – and thus time-consuming – methods when this can be achieved much more easily by use of an enzyme. Samuel recommended the content of chemistry currucila to be geared to Life Sciences; some examples 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 *et al.*, 1971; 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). 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 to some extent the progress in research and thereby help to keep teaching up-to-date.

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