

# Biocatalysis

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## INTRODUCTION

Biocatalysis may be broadly defined as the use of enzymes or whole cells as biocatalysts for industrial synthetic chemistry. They have been used for hundreds of years in the production of alcohol via fermentation, and cheese via enzymatic breakdown of milk proteins. Over the past few decades, major advances in our understanding of the protein structure–function relationship have increased the range of available biocatalytic applications. In particular, new developments in protein design tools such as rational design and directed evolution have enabled scientists to rapidly tailor the properties of biocatalysts for particular chemical processes. Rational design involves rational alterations of selected residues in a protein to cause predicted changes in function, whereas directed evolution, sometimes called irrational design, mimics the natural evolution process in the laboratory and involves repeated cycles of generating a library of different protein variants and selecting the variants with the desired functions (see the entry “Protein Design”). Enzyme properties such as stability, activity, selectivity, and substrate specificity can now be routinely engineered in the laboratory. Presently, approximately 100 different biocatalytic processes are implemented in pharmaceutical, chemical, agricultural, and food industries.<sup>[1]</sup> The products range from research chemicals to commodity chemicals and the number of applications continue to grow very rapidly. In spite of these successes, however, the vast potential of biocatalysis has yet to be fully realized.

In this entry, we briefly outline the scope of biocatalysis and discuss its advantages and disadvantages as compared to chemical catalysis. We then review such topics as enzyme and whole-cell based biocatalysis, biocatalysts used in nonaqueous media, biocatalyst immobilization, discovery and engineering of novel enzymes, and hybrid approaches combining chemical and biological synthesis. An overview of the six general classifications of enzymes along with their relative use in industry is discussed. Selected industrial applications of whole-cell based biocatalysis including the production of lactic acid and 1,3-propanediol are also studied.

## THE SCOPE OF BIOCATALYSIS

### Advantages and Disadvantages of Biocatalysis vs. Chemical Catalysis

Similar to other catalysts, biocatalysts increase the speed in which a reaction takes place but do not affect the thermodynamics of the reaction. However, they offer some unique characteristics over conventional catalysts (Table 1). The most important advantage of a biocatalyst is its high selectivity. This selectivity is often chiral (i.e., stereo-selectivity), positional (i.e., regio-selectivity), and functional group specific (i.e., chemo-selectivity). Such high selectivity is very desirable in chemical synthesis as it may offer several benefits such as reduced or no use of protecting groups, minimized side reactions, easier separation, and fewer environmental problems. Other advantages, like high catalytic efficiency and mild operational conditions, are also very attractive in commercial applications.

The characteristics of limited operating regions, substrate or product inhibition, and reactions in aqueous solutions have often been considered as the most serious drawbacks of biocatalysts. Many of these drawbacks, however, turn out to be misconceptions and prejudices.<sup>[2,3]</sup> For example, many commercially used enzymes show excellent stability with half-lives of months or even years under process conditions. In addition, there is an enzyme-catalyzed reaction equivalent to almost every type of known organic reaction. Many enzymes can accept non-natural substrates and convert them into desired products. More importantly, almost all of the biocatalyst characteristics can be tailored with protein engineering and metabolic engineering methods (refer to the section Biocatalyst Engineering and see also the entry “Protein Design”) to meet the desired process conditions.

Biocatalytic processes are similar to conventional chemical processes in many ways. However, when considering a biocatalytic process one must account for enzyme reaction kinetics and enzyme stability for single-step reactions, or metabolic pathways for multiple-step reactions.<sup>[4]</sup> Fig. 1 shows the key steps

**Table 1** Advantages and disadvantages of biocatalysis in comparison with chemical catalysis

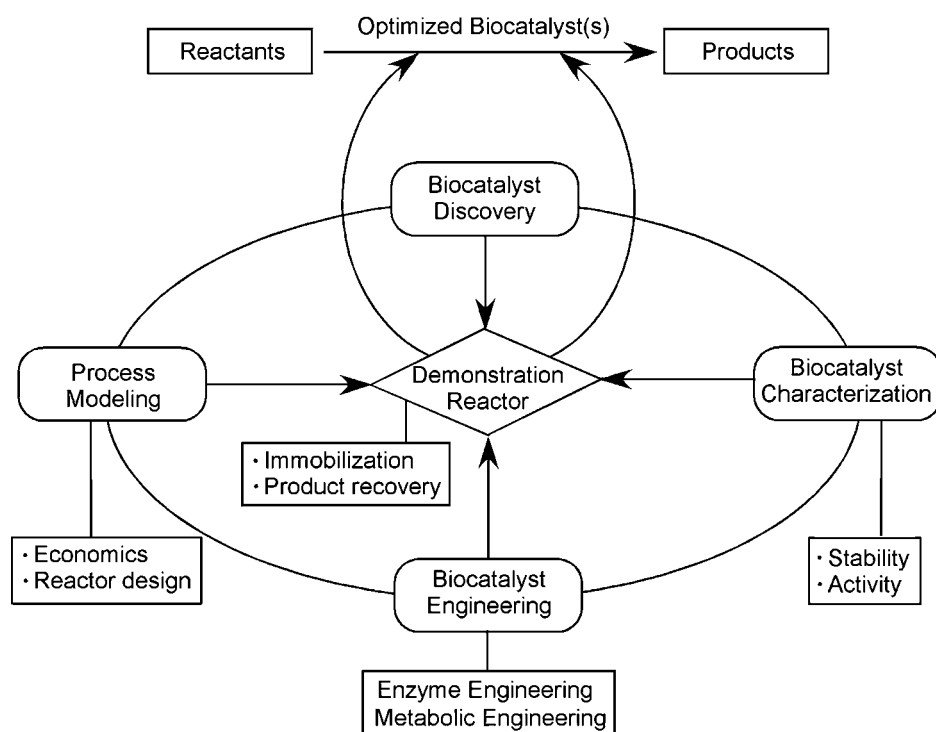
Advantages	Disadvantages
Generally more efficient (lower concentration of enzyme needed)	Susceptible to substrate or product inhibition
Can be modified to increase selectivity, stability, and activity	Solvent usually water (high boiling point and heat of vaporization)
More selective (types of selectivity: chemo-selectivity, regio-selectivity, diastereo-selectivity, and enantio-selectivity)	Enzymes found in nature in only one enantiomeric form
Milder reaction conditions (typically in a pH range of 5–8 and temperature range of 20–40°C)	Limiting operating region (enzymes typically denatured at high temperature and pH)
Environment friendly (completely degraded in the environment)	Enzymes can cause allergic reactions

(From Ref.<sup>[2]</sup>)

in the development of a biocatalytic process. It usually starts with the identification of a target reaction, followed by biocatalyst discovery, characterization, engineering, and process modeling. In many cases, biocatalyst engineering is the most time-consuming step, often involving two major approaches: rational design and directed evolution. In addition to biocatalyst development, product isolation is an important step. The overall process economics depends on all these factors, which needs to be demonstrated in a pilot-scale plant before scale-up. Biocatalysts can constitute a significant portion of the operating budget; however, their cost can be reduced by reusing them when immobilized (refer to the section Biocatalyst Immobilization).

### Enzyme Based Biocatalysis vs. Whole-Cell Biocatalysis

Both isolated enzymes and whole cells can be used as biocatalysts. Compared to whole cells, isolated enzymes offer several benefits, including simpler reaction apparatus, higher productivity owing to higher catalyst concentration, and simpler product purification.<sup>[2]</sup> Until recently, only enzymes that were abundantly produced by cells could be used in industrial applications. Now it is possible to produce large amounts of an enzyme through the use of recombinant DNA technology. In brief, the DNA sequence encoding a given enzyme is cloned into an expression vector and transferred into a production host such as

**Fig. 1** Flowchart of the development of a biocatalytic process.

*Escherichia coli* or *Saccharomyces cerevisiae* for gene expression. The overexpressed enzymes are purified from the cell extracts based on their chemical and physical properties. The most commonly used enzyme purification techniques include electrophoresis, centrifugation, and chromatography. Centrifugation separates enzymes based on their differences in mass or shape, whereas electrophoresis separates enzymes based on their differences in charge. Liquid chromatography separates enzymes based on their differences in charge (ion-exchange chromatography), in mass (gel filtration chromatography), or in ligand-binding property (affinity chromatography).

The whole-cell biocatalysis approach is typically used when a specific biotransformation requires multiple enzymes or when it is difficult to isolate the enzyme. A whole-cell system has an advantage over isolated enzymes in that it is not necessary to recycle the cofactors (nonprotein components involved in enzyme catalysis). In addition, it can carry out selective synthesis using cheap and abundant raw materials such as cornstarches. However, whole-cell systems require expensive equipment and tedious work-up because of large volumes, and have low productivity. More importantly, uncontrolled metabolic processes may result in undesirable side reactions during cell growth. The accumulation of these undesirable products as well as desirable products may be toxic to the cell, and these products can be difficult to separate from the rest of the cell culture. Another drawback to whole-cell systems is that the cell membrane may act as a mass transport barrier between the substrates and the enzymes.

### Nonaqueous Biocatalysis

Historically, enzymes have been used extensively in aqueous media. Enzymes are well suited to their natural aqueous environment; however, biotransformations in industrial synthesis often involve organic molecules insoluble in water. More importantly, because of its high boiling point and high heat of vaporization, water is usually the least desired solvent of choice for most organic reactions. Thus, shifting enzymatic reactions from an aqueous to an organic medium is highly desired.

Over the past 15 yr, studies have shown that enzymes can work in organic solvents.<sup>[5]</sup> However, the enzymatic activity is quite low in an organic solvent compared to that in water. Recent advances in protein engineering and directed evolution have aided in the development of enzymes that show improved activity in organic solvents. Progress has also been made in developing simple, scalable, and low-cost techniques to produce highly active biocatalyst preparations for use

in organic solvents.<sup>[6]</sup> One such method improves enzyme activity in organic solvents by lyophilizing (freeze-drying) an aqueous biocatalyst solution in the presence of organic and inorganic molecules called excipients. These excipients include nonbuffer salts, crown ethers, cyclodextrins, and solid-state buffers.<sup>[7]</sup> Some remarkable results have also been achieved by using ionic liquids as solvents in biocatalytic reactions.<sup>[8]</sup>

### Biocatalyst Immobilization

Immobilization is the process of adhering biocatalysts (isolated enzymes or whole cells) to a solid support. The solid support can be an organic or inorganic material, such as derivatized cellulose or glass, ceramics, metallic oxides, and a membrane. Immobilized biocatalysts offer several potential advantages over soluble biocatalysts, such as easier separation of the biocatalysts from the products, higher stability of the biocatalyst, and more flexible reactor configurations. In addition, there is no need for continuous replacement of the biocatalysts. As a result, immobilized biocatalysts are now employed in many biocatalytic processes.

More than one hundred techniques for immobilizing enzymes have been developed which can be divided into five major groups summarized in Table 2.<sup>[9]</sup> Adsorption of the enzyme onto a surface is the easiest and the oldest method of immobilization. Entrapment and cross-linking tend to be more laborious enzyme fixation methods, but they do not require altering the enzyme as much as other techniques. The formation of the covalent linkage often requires harsh conditions, which can result in a loss of activity because of conformational changes of the enzyme. It is important to note that most of these techniques can also be used to immobilize whole cells. In addition, although these types of immobilization are considered to be relatively old and well established, the emerging field of nanotube biotechnology has created another possible means of immobilizing biocatalysts.<sup>[10]</sup>

### Biocatalyst Discovery: Sources and Techniques

Traditionally, potentially commercial enzymes are identified by screening micro-organisms, which are frequently isolated from extreme environments, for biocatalytic activity. Commercial enzymes are selected by probing libraries of related enzymes for a range of properties, including activity, substrate specificity, stability over a temperature range, enantio-selectivity, or compatibility under various physical and chemical conditions. Unfortunately, most of the commercially viable enzymes have been isolated in only a few microbial species such as *Bacillus* and *Pseudomonas* because

**Table 2** Methods of enzyme immobilization

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A. Covalent attachment	Isolated enzymes usually attached through amino or carboxyl groups to a solid support Variety of supports such as porous glass, cellulose, ceramics, metallic oxides
B. Adsorption	Ion-exchangers frequently used in industry because of simplicity Industrial applications include anion-exchangers diethylaminoethyl cellulose (DEAE-cellulose) and the cation-exchanger carboxymethyl cellulose (CM-cellulose)
C. Entrapment in polymeric gels	Enzyme becomes trapped in gel volume by changing temperature or adding gel-inducing chemical Enzymes may be covalently bound to gel (for instance, polyacrylamide cross-linked with <i>N,N'</i> -methylenebisacrylamide) or noncovalently linked (calcium alginate)
D. Intermolecular cross-linking	Enzyme cross-linked with bifunctional reagents Popular cross-linkers are glutaraldehyde, dimethyl adipimidate, dimethyl suberimidate, and aliphatic diamines
E. Encapsulation	Enzymes enveloped in semipermeable membrane, which allows low molecular weight substrates and products to pass through the membrane Enclosed in a variety of devices: hollow fibers, cloth fibers, microcapsules, film

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(From Ref.<sup>[9]</sup>.)

of the limitations in micro-organism cultivation techniques.<sup>[11]</sup> It has been widely acknowledged that the majority of microbial species (up to 99%) have never been cultivated and thus have never been investigated. To access this vast untapped microbial diversity, several companies such as Diversa Corporation (San Diego, California, U.S.A.) and TerraGen Discovery (Vancouver, British Columbia, Canada) have successfully developed modern bioprospecting techniques such as multiple metagenome cloning to isolate novel industrial enzymes.<sup>[12]</sup>

New methods for exploring natural biodiversity have been greatly facilitated by high-throughput screening technologies and robust expression in recombinant organisms. Recombinant DNA technology makes it possible to produce enzymes at levels 100-fold greater than native expression and allows expression of genes from organisms that cannot be cultured. Although some problems may be resolved by screening larger libraries of DNA, this may not be the most efficient or expedient method of obtaining a viable biocatalyst. A more efficient means of obtaining a good biocatalyst may involve engineering the catalyst itself using various protein engineering and metabolic engineering techniques (refer to the section Biocatalyst Engineering and see also the entry “Protein Design”).

## Biocatalyst Engineering

Nature has supplied us with a vast array of biocatalysts capable of catalyzing numerous biological reactions. Unfortunately, naturally occurring biocatalysts are often not optimal for many specific industrial applications,

such as low stability and activity. Moreover, naturally occurring biocatalysts may not catalyze the reaction with the desired non-natural substrates or produce the desired products. To address these limitations, molecular techniques have been developed to create improved or novel biocatalysts with altered industrial operating parameters. It should be noted that, for enzyme based biocatalysts, many molecular techniques have been developed for engineering enzymes with novel or improved characteristics. Readers are referred to the entry “Protein Design.” In this section, we mainly discuss the molecular techniques used for whole-cell based biocatalyst engineering, or metabolic engineering.

Metabolic engineering is a rapidly growing area with great potential to impact biocatalysis.<sup>[13]</sup> It has been broadly defined as “the directed improvement of product formation or cellular properties through modifications of specific biochemical reaction(s) or the introduction of new one(s) with the use of recombinant DNA technology.”<sup>[14]</sup> In an industrial context, the ultimate goal of metabolic engineering is the development of optimal biocatalysts. In the past two decades, metabolic engineering has been successfully used to engineer micro-organisms to produce a wide variety of products, including polymers, aromatics, carbohydrates, organic solvents, proteins, antibiotics, amino acids, and organic acids. According to the approach taken or the aim, these applications can be classified into seven groups: 1) expression of heterologous genes for protein production; 2) extension of the range of substrate for cell growth and product formation; 3) design and construction of pathways for the production of new products; 4) design and

construction of pathways for degradation of xenobiotics; 5) engineering of cellular physiology for process improvement; 6) elimination or reduction of by-product formation; and 7) improvement of yield or productivity.<sup>[15]</sup> Several of these applications have been implemented at industrial-production scale (refer to the section Industrial Applications of Whole-Cell Based Biocatalysis) and the number of applications should continue to grow. In particular, with the recent advances in genomics, proteomics, and bioinformatics, many new genes and pathways will be discovered and the regulation of metabolic network will also be better understood, all of which will accelerate the development of more commercially viable bioprocesses through metabolic engineering.

### Hybrid Approaches Combining Chemical Synthesis and Biocatalysis

Biocatalysts exhibit exquisite catalytic efficiency that is often unmatched by conventional catalysis. Nonetheless, conventional organic synthesis will likely remain the staple of the chemical and pharmaceutical industries. In the future, the integration of these two approaches will probably offer the optimal route for industrial synthesis. An illustration of this principle can be found in the selective deprotection of reactive functional groups. Enzymes are unique deprotecting tools for combinatorial synthesis because of their remarkable selectivity and ability to operate under mild reaction conditions. A recent example is the synthesis of long multiply lipidated peptides containing various side-chain functional groups.<sup>[16]</sup> In this study, penicillin acylase was used for selective N-deprotection of a highly labile S-palmitoylated oligopeptide. After removal of the protecting group, the S-palmitoylated oligopeptide was used as a building block in further synthetic steps.

## INDUSTRIAL APPLICATIONS OF ENZYME BASED BIOCATALYSIS

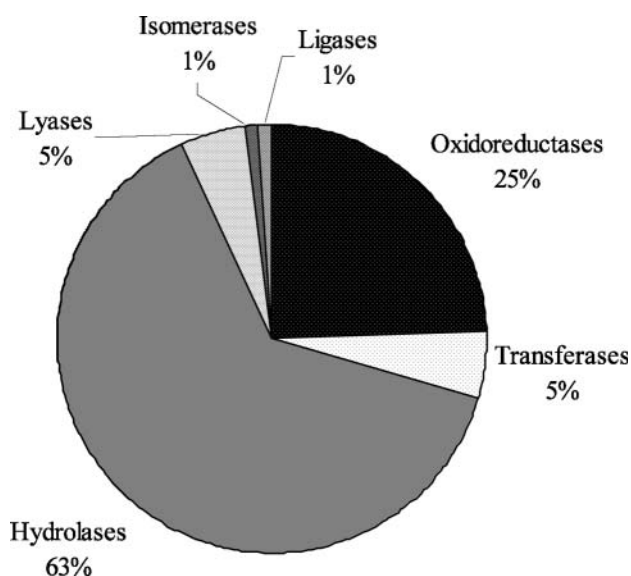
With the rapid technical developments in gene discovery, optimization, and characterization, enzymes have been increasingly used as biocatalysts. According to the International Union of Biochemistry and Molecular Biology (IUBMB) nomenclature system, all enzymes are classified into six classes on the basis of the general type of reactions that they catalyze (Table 3). Within each class are subclasses and the enzymes themselves. The result is an ordered system of enzymes and the reaction(s) that each catalyzes. It is important to note that, in biological processes, every class of enzyme is utilized in the cell to a large extent. However, this is not the same in industrial processes, where certain classes of enzymes are used more often than others. As shown in Fig. 2, most of the enzymes that have been used as biocatalysts in industry are hydrolases (~65%), even though oxidoreductases are typically much more useful than hydrolases as catalysts. The utility of an enzyme class depends on the relative commercial importance of the products that each enzyme produces, the accessibility of the enzymes, and the specific characteristics of the enzymes (e.g., stability, activity, and selectivity).

### Oxidoreductases

Oxidoreductases catalyze oxidation and reduction reactions that occur within the cell. They are very appealing for industrial uses because of the reactions that they are able to catalyze. However, they often need expensive cofactors such as nicotinamide adenine dinucleotides (e.g., NAD<sup>+</sup>/NADH) and flavines (e.g., FAD/FADH<sub>2</sub>) in the reactions. In fact, nicotinamide adenine dinucleotides are required by about 80% of oxidoreductases. Fortunately, several NAD(H)

**Table 3** Classification of enzymes

Enzymes	Type of reactions	Representative subclasses
Oxidoreductases	Catalyze the transfer of hydrogen or oxygen atoms or electrons from one substrate to another	Oxidases, oxygenases, peroxidase, dehydrogenases
Transferases	Catalyze the group transfer reactions	Glycosyltransferases, transketolases, methyltransferases, transaldolases, acyltransferases, transaminases
Hydrolases	Catalyze hydrolytic reactions	Esterases, lipases, proteases, glycosidases, phosphatases
Lyases	Catalyze the nonhydrolytic removal of groups	Decarboxylases, aldolases, ketolases, hydratases, dehydratases
Isomerases	Catalyze isomerization reactions	Racemases, epimerases, isomerases
Ligases	Catalyze the synthesis of various types of bonds with the aid of energy-containing molecules	Synthetases, carboxylases

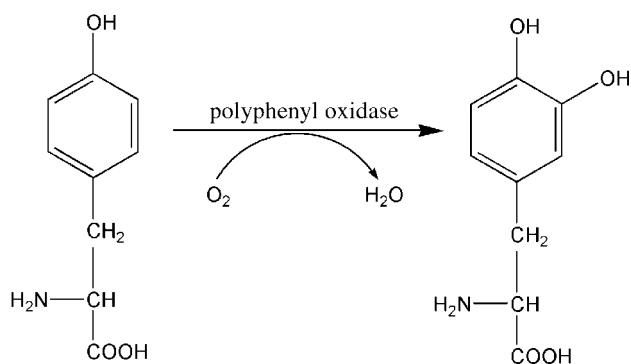


**Fig. 2** The relative use of enzyme classes in industry. (From Ref.<sup>[2]</sup>.)

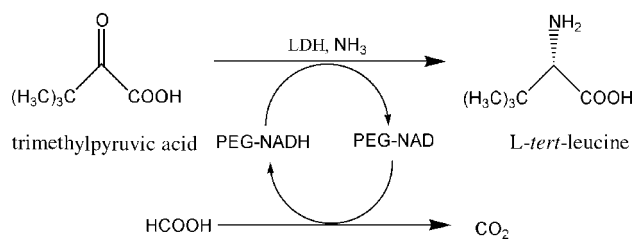
regeneration systems have been developed, the most widely used being the formate/formate dehydrogenase (FDH) system.<sup>[17]</sup>

An example of a pharmaceutical synthesis reaction involving an oxidoreductase is the synthesis of 3,4-dihydroxyphenyl alanine (DOPA).<sup>[2]</sup> 3,4-Dihydroxyphenyl alanine is a chemical used in the treatment of Parkinson's disease. The industrial process that synthesizes DOPA utilizes the oxidoreductase polyphenol oxidase. As shown in Fig. 3, the monohydroxy compound is oxidized by the regio-specific addition of a hydroxyl group. It is worth mentioning that epinephrine (adrenaline) can also be synthesized by a similar reaction path using the same enzyme.<sup>[2]</sup>

Another example is the use of leucine dehydrogenase coupled with FDH for the reductive amination of trimethylpyruvate to *L-tert-leucine* (Fig. 4). The whole



**Fig. 3** Enzymatic synthesis of 3,4-dihydroxyphenyl alanine (DOPA).



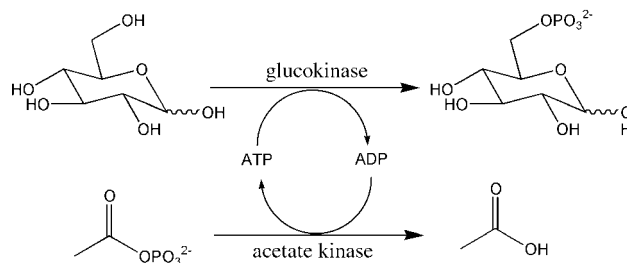
**Fig. 4** Enzymatic synthesis of *L-tert-leucine*.

process is carried out in a membrane reactor in which the cofactor  $\text{NAD}^+$  is regenerated by FDH. This process has now reached ton-scale production at Degussa (Germany).<sup>[18]</sup>

### Transferases

Transferases catalyze the transfer of functional groups such as methyl, hydroxymethyl, formal, glycosyl, acyl, alkyl, phosphate, and sulfate groups by means of a nucleophilic substitution reaction. They are not widely used in industrial processes; however, there are a few examples of industrial processes that utilize transferases.

A classical example of industrial application of transferases is the use of various glycosyltransferases for the synthesis of oligosaccharides. Oligosaccharides and polysaccharides are important classes of naturally occurring compounds, which play vital roles in cellular recognition and communication processes.<sup>[19]</sup> Because of the required use of many protection and deprotection groups, chemical synthesis of complex oligosaccharides represents a daunting challenge in synthetic organic chemistry. By contrast, enzymatic synthesis of oligosaccharides by glycosyltransferases requires very few protection and deprotection steps because of the high regio- and stereoselectivity of glycosyltransferases, thus offering an attractive alternative.<sup>[2]</sup> Another example is the use of a glucokinase (a transferase) in combination with an acetate kinase for the production of glucose-6-phosphate (Fig. 5). This process is carried out in multikilogram scale by the Japanese company Unitika.<sup>[1]</sup>



**Fig. 5** Enzymatic synthesis of glucose-6-phosphate.

## Hydrolases

Hydrolases catalyze the addition of water to a substrate by means of a nucleophilic substitution reaction. Hydrolases (hydrolytic enzymes) are the biocatalysts most commonly used in organic synthesis. They have been used to produce intermediates for pharmaceuticals and pesticides, and chiral synthons for asymmetric synthesis. Of particular interest among hydrolases are amidases, proteases, esterases, and lipases. These enzymes catalyze the hydrolysis and formation of ester and amide bonds.

Lipases can hydrolyze triglycerides into fatty acids and glycerol. They have been used extensively to produce optically active alcohols, acids, esters, and lactones by kinetic resolution. Lipases are unique, in that they are usually used in two-phase systems. A classic example is the use of a lipase for the production of (*S,R*)-2,3-*p*-methoxyphenylglycyclic acid, an intermediate for diltiazem. In this process, methyl-*p*-methoxyphenylglycidate is stereospecifically hydrolyzed by a lipase immobilized in a hollow fiber membrane reactor. The enzyme is located at the interfacial layer between an organic and an aqueous phase.<sup>[1]</sup>

Proteases such as  $\alpha$ -chymotrypsin, papain, and subtilisin are also useful biocatalysts for regio-selective or stereoselective hydrolytic biotransformations. For example, dibenzyl esters of aspartic and glutamic acid can be selectively deprotected at the 1-position by subtilisin-catalyzed hydrolysis (Fig. 6).<sup>[2]</sup> In addition,  $\alpha$ -chymotrypsin is used in the kinetic resolution of  $\alpha$ -nitro- $\alpha$ -methyl carboxylates, which results in *L*-configured enantiomers of the unhydrolyzed esters with high optical purity (>95% e.e.).<sup>[2]</sup>

## Lyases

Lyases are the enzymes responsible for catalyzing addition and elimination reactions. Lyase-catalyzed reactions involve the breaking of a bond between a carbon atom and another atom such as oxygen, sulfur, or another carbon atom. They are found in cellular processes, such as the citric acid cycle, and in organic synthesis, such as in the production of cyanohydrins.<sup>[2]</sup>

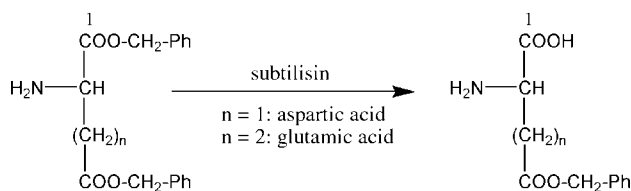


Fig. 6 Regio-selective ester-hydrolysis catalyzed by subtilisin.

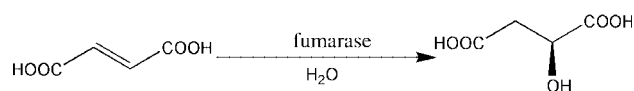


Fig. 7 Enzymatic synthesis of (*S*)-malic acid.

Several industrial processes using lyases as catalysts have been reported. Perhaps the most prominent lyase-catalyzed process is the production of acrylamide from acrylonitrile. This process is carried out by the Nitto Chemical Company of Japan at a scale of more than 40,000 tons per year.<sup>[20]</sup> Another example is the use of a fumarase for the production of (*S*)-malic acid from fumaric acid. As shown in Fig. 7, a water molecule is added to the double bond in fumarate by means of an addition reaction. The result is a cleavage of the carbon-carbon double bond, and a formation of a new carbon-oxygen bond. A third example is biocatalytic production of a cyanohydrin from a ketone. This reaction is catalyzed by a lyase called oxynitrilase. It consists of the cleavage of one carbon-oxygen bond, and the addition of a HCN molecule. The chirality of the product is based on the form of the enzyme used (*R*-oxynitrilase or *S*-oxynitrilase).<sup>[2]</sup>

## Isomerases

Isomerases catalyze isomerization reactions such as racemization and epimerization. They have not been used in many industrial applications. However, one of the most successful enzyme based biocatalytic processes involves an isomerase: the use of glucose isomerase for the production of high-fructose corn syrup (HFCS) (Fig. 8). High-fructose corn syrup is used as an alternative sweetener to sucrose in the food and beverage industry. The isomerization of glucose to HFCS on an industrial scale is carried out in continuous fixed-bed reactors using immobilized glucose isomerases. The total amount of HFCS produced by glucose isomerase exceeds a million tons per year.<sup>[21]</sup>

## Ligases

Ligases catalyze reactions that involve the creation of chemical bonds with nucleotide triphosphates. They

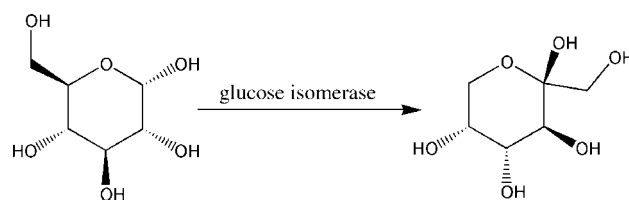


Fig. 8 Enzymatic synthesis of high-fructose corn syrup.

are important in certain cellular processes, such as connecting nucleotides in DNA replication. However, similar to isomerases, ligases have very few industrial applications.<sup>[2]</sup> It is important to note that DNA ligases are essential tools in recombinant DNA technology and are used almost in every biology-related laboratory.

## INDUSTRIAL APPLICATIONS OF WHOLE-CELL BASED BIOCATALYSIS

Whole-cell based biocatalysis utilizes an entire micro-organism for the production of the desired product. One of the oldest examples for industrial applications of whole-cell biocatalysis is the production of acetic acid from ethanol with an immobilized *Acetobacter* strain, which was developed nearly 200 yr ago.<sup>[1]</sup> The key advantage of whole-cell biocatalysis is the ability to use cheap and abundant raw materials and catalyze multistep reactions. Recent advances in metabolic engineering have brought a renaissance to whole-cell biocatalysis. In the following sections, two novel industrial processes that utilize whole-cell biocatalysis are discussed with emphasis on the important role played by metabolic engineering.

### Lactic Acid

L-lactic acid has long been used as a food additive and has recently received great attention because it can be used as an important feedstock for the production of other chemicals such as polylactic acid (PLA), acetaldehyde, polypropylene glycol, acrylic acid, and penta-dione.<sup>[22]</sup> Among them, PLA is the most important product as it can be used to manufacture thermoformed containers, packaging, nonwovens, paper-coated articles, and film products.<sup>[23]</sup> Lactic acid can be produced from sucrose, whey (lactose), and maltose or dextrose from hydrolyzed starch using *Lactobacillus* strains.

Compared to other polymeric materials such as polyethylenes, polylactic acid has several advantages including an increased hydrophilicity, resistance to ultraviolet light, ability to be dyed with dispersion dyes, a range of melting temperatures between 120°C and 170°C, and low flammability and smoke generation. Most importantly, polylactic acid is biodegradable and is derived from renewable resources, utilizing energy from the sun and lowering the fossil fuel dependence for production.<sup>[23]</sup>

In recognition of the superior properties and the huge potential market of polylactic acid, Cargill Inc. and The Dow Chemical Company started a joint venture Cargill Dow LLC to produce lactic acid using

fermentation (whole-cell biocatalysis) several years ago. A production plant was built in Blair, NE, in 2001 and it now produces 140,000 metric tons of polylactic acid per year. It is predicted that the eventual cost of polylactic acid will be between \$0.50 and \$0.75 per pound (<http://www.cargilldow.com>).

One of the drawbacks in the current commercial fermentation process is that the predominant form of the product is the deprotonated lactate rather than lactic acid, requiring more expensive and wasteful product purification steps. This is because the *Lactobacillus* fermentation operates at a minimum pH of 5.0–5.5 which is above the  $pK_a$  of lactic acid (3.87). To overcome this limitation, a powerful strain improvement method, genome shuffling, was used to improve the acid tolerance of a poorly characterized industrial strain of *Lactobacillus*.<sup>[24]</sup> A population of strains with subtle improvement in pH tolerance was isolated using classical strain improvement methods such as chemostats, and were then shuffled by recursive pool-wise protoplast fusion to create mutant strains that grow at substantially lower pH than does the wild-type strain.

### 1,3-Propanediol

1,3-Propanediol is an intermediate that is widely used in the synthesis of polyesters and polyurethanes. Polymers based on 1,3-propanediol are very useful in the carpet and textile industry because of their good light stability and biodegradability.<sup>[25]</sup> The conventional methods for producing 1,3-propanediol rely on petroleum derivatives and are quite capital intensive and/or generate waste streams containing environmental pollutants. Thus, the use of micro-organisms to produce 1,3-propanediol from glucose represents an attractive alternative.

Both the biological production of 1,3-propanediol from glycerol and that of glycerol from glucose have been known for many years.<sup>[13]</sup> However, there is no single micro-organism that could convert basic carbon sources such as glucose to the desired 1,3-propanediol end-product. Such a micro-organism is highly desired in the process as it requires less energy input and uses an inexpensive starting material.

A team of researchers from DuPont and Genencor has successfully used metabolic engineering techniques to engineer such a micro-organism. The conversion of glucose to 1,3-propanediol requires the combination of two natural pathways: glucose to glycerol and glycerol to 1,3-propanediol. The best natural pathway for the production of glycerol from glucose was found in the yeast *Saccharomyces cerevisiae*, which consists of two enzymes: dihydroxyacetone-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase. The best natural

pathway for production of 1,3-propanediol from glycerol was found in *Klebsiella pneumoniae*, which consists of glycerol dehydratase and 1,3-propanediol dehydrogenase. The genes encoding these two natural pathways were cloned and expressed in *E. coli*. *E. coli* was chosen as the production strain because it has been used in large-scale production on an industrial level, it has many genetic tools, and its metabolism and physiology are well characterized. This engineered *E. coli* was found to produce over 120 g/L of 1,3-propanediol in 40 hr fed-batch fermentation.<sup>[13]</sup>

## CONCLUSIONS

Biocatalysis has become an important tool for industrial chemical synthesis and is on the verge of significant growth. In the past several decades, many biocatalytic processes have been implemented to produce a wide variety of products in various industries. Most of them use naturally occurring enzymes or micro-organisms as catalysts. With the help of innovative biocatalyst discovery methods and advances in protein engineering and metabolic engineering, the time and cost of developing new biocatalysts can be reduced significantly. Most importantly, the biocatalysts can be readily tailored to their specific applications and process conditions through protein engineering and metabolic engineering. It is possible that in the future they can be rationally designed to act specifically in any chemical reaction of interest, fulfilling the holy grail of catalysis: catalysis by design. In addition, the use of biocatalysts in organic solvents in combination with the integration of biocatalysis and chemical catalysis will continue to broaden the scope of the applications of biocatalysts. New advances in genomics, proteomics, and bioinformatics will fuel the development of biocatalysis as an integral part of industrial catalysis.

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