Molecular and Engineering Perspectives of the Biocatalysis Interface to Chemical Synthesis*

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Review
Received: January 11, 2011
Accepted: March 14, 2011

The sustainable use of limited resources by nature to provide target molecules with biocatalytic reactions continues to be a role model for chemical synthesis. The application of biocatalysts to functional group transformations is shaped by the various parallel influences like e.g. the search for selectivity, the shift from fossil-based to biobased raw materials and the economy of molecular transformations like atom economy and step economy. As safety, health and environment issues are key drivers for process improvements in the chemical industry, the development of reactions or pathways replacing hazardous reagents is another major factor determining the sequence of molecular transformations from raw material to product.

Biocatalyst production technologies and integrated process engineering have been instrumental in the establishment of biocatalytic reaction steps in chemical synthesis. The inherent properties of biocatalysts make them the privileged catalysts for highly selective asymmetric molecular transformations like e.g. hydrolysis reactions, oxidation reactions, carbon-carbon bond formation reactions as well as molecular unit transfer reactions. The universe of six enzyme classes provides a tremendous goldmine for discovering improved versions of enzymes with known functions as well as for finding completely novel enzymes. With the growing collection of biocatalytic reactions, the retrosynthetic thinking from chemical synthesis can be applied to biocatalysis as well.

Once the feasibility of a biocatalytic reaction has been proven, up- and downscaling experiments have been useful for engineering the most adequate process design. In the case of the first large-scale biocatalytic Baeyer-Villiger oxidation, the debottlenecking of the substrate feed and product recovery, final purification and overcoming thermodynamic limitations have been essential in establishing bioprocesses with high yields of enantiopure products. These downscaling experiments in conjunction with new analytical techniques have proven useful also in the case of asymmetric synthesis of natural compounds. Spatial and temporal organisation of biocatalysts, reactants or products is another interesting engineering option for biocatalytic process design.

The interdisciplinary character of the dead ends and locks between chemistry, biology and engineering requires investigations of the interfaces. Communication across scientific and technological disciplines including the value creation perspective is important for the development of a better synthesis for the final product-in-the-bottle. Whether the successful problem solution will come from the engineering of substrates, reaction media, process conditions or from the search for better and new enzymes, progress in the understanding of the molecular mechanisms of enzyme action will be key for the further development of the science of synthesis with its challenges towards the more difficult and more complex target molecules.

Key words:
Atom economy, step economy, redox economy, enzymatic resolution, asymmetric synthesis, biotransformation, biocatalysts, oxidoreductases, transferases, hydrolases, lyases, biocatalytic process design, downstream processing, scalability

Introduction

The knowledge in the preparation of natural and synthetic compounds has led to an enormous number of different molecular structures for an increasing number of applications. The growing human population is requiring larger amounts of products and intermediates, which have been and continue to be supplied by scaling up manual syntheses from small volumes to industrial large-scale and highly automated and controlled productions. While the science of synthesis has contributed spectacular advances in the high-yield preparation of the

*Based on an Invited Lecture at the XXI Croatian Meeting of Chemists and Chemical Engineers, Trogir, April 19–22, 2009.
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most complex molecular structures from simple building blocks or intermediates, the applications on industrial large-scale or in biological systems remind us of the many challenges, bottlenecks and dead ends which may be encountered on the route from educt to product:

1) Protection of the macroenvironment (humans, environment, product) or the microenvironment (other functional groups of the molecule, other molecular components or parts of the cell) may represent challenging tasks

2) Available known reactions may not be selective enough

3) No direct synthetic route from the starting material to the product may be known

4) Too many steps and detours for the intended synthetic route may be required or a key reaction step may turn out to be a dead-end

5) Waste growth in relation to the growth of the desired product may create problems due to unfavourable product/waste-ratios.

Waste accumulation with each reaction step is a common feature of production processes in chemical synthesis, illustrated in Fig. 1 schematically from starting material to product via one or more intermediates. Therefore, increased attention to waste is of prime importance, in addition to the traditional synthetic criteria such as product yield and purity. If the cumulated costs of waste disposal for each non-sustainable reaction step in Fig. 1 approach the market value of the product, waste

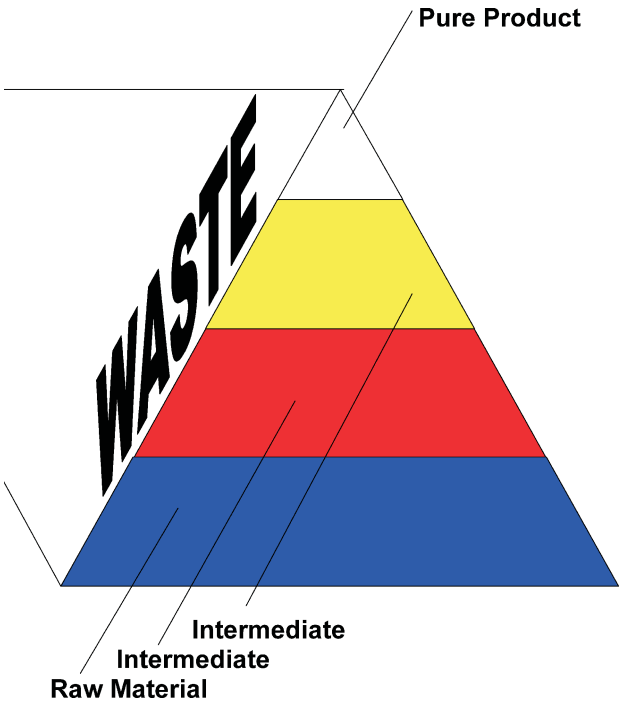


Fig. 1 – *Waste accumulation from starting material to the yield of pure product*

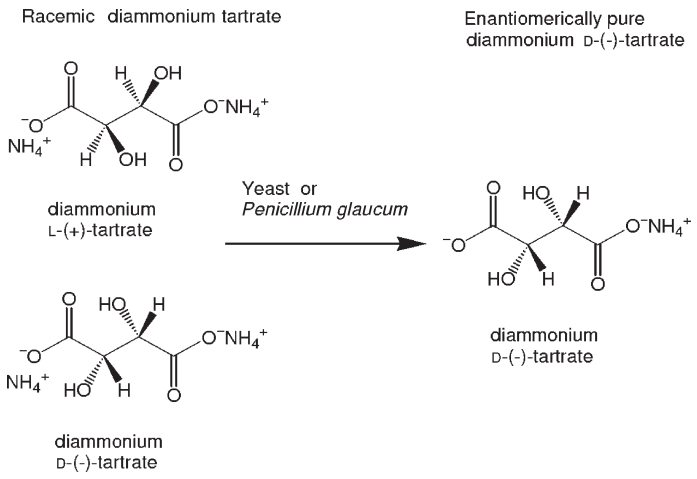
minimization becomes an economic necessity for the viability of manufacturing processes worldwide. This has led to the proposal of the Environmental Factor (short E Factor) by Roger Sheldon[1] in the late 1980s for assessing the environmental impact of manufacturing processes. The E factor concept (E equals kg waste divided by kg product), as an overall measure for the amount of waste produced in a synthesis compared with the amount of isolated product, has become an important parameter driving new synthetic developments.[1] The economy of a synthetic scheme on a molecular scale can be viewed from different perspectives, but all aim at making the best use of materials and minimizing the waste. This is a strategy that biological cells use with the help of nature's catalysts in order to avoid the scaling of waste with the increasing amount of product as in stoichiometric reactions in chemical synthesis. From the single reaction step perspective, selective transformations with high atom economy continue to be a main focus of research in the science of synthesis.[2]

The attention to the overall number of steps in a synthesis scheme aims at achieving a high step economy by reducing the number of steps and replacing protection-transformation-deprotection schemes by direct transformations.[3] Further waste minimizations in synthetic schemes involve the redox economy[4] and reducing the use of auxiliary reagents and solvents in product isolations and purifications. As waste is still accumulating with many existing technologies at present, searching for waste degradation to harmless compounds or for waste conversion into new useful products continues to be relevant and may help close the path of carbon. For producing useful compounds as well as for converting waste into harmless or even useful materials rather than just waste disposal, catalysis has been and continues to be the key to economical, energy-saving and environmentally benign chemical transformations.[5,6]

The early history of biocatalysis is closely related to the preparation of alcoholic beverages like beer and wine. A scientific milestone in the 19th century has been the theoretical concept that chemical transformations of living organisms in all forms of life, not only microorganisms, could be related back to the work of specific substances which would accelerate reactions and function in a similar way as cellular machines. The experimental discovery of enzymes as these specific substances acting like molecular machines with the ability to accelerate chemical reactions in living organisms has sparked tremendous interest until the present day and the scientific discipline of biochemistry originated with the goal to isolate enzymes from biological cells and determine their functioning on a mo-

lecular level. As enzymes within a biological cell perform the required molecular transformations with excellent selectivity, the waste is minimized and product yield maximized. This is due to an enormous amount of work that enzymes have achieved today a remarkable success in catalyzing useful reactions in chemical synthesis.[7–10]

The importance of both the molecular and engineering aspects of the application of biocatalysis in the synthesis of chemicals has already been demonstrated by Louis Pasteur in 1858 in the successful microbial resolution of racemic tartaric acid, illustrated in Fig. 2, combining the selective microbial degradation of one enantiomer with the crystallization of the remaining enantiomer.[11,12] The understanding of biocatalysis as a key to the chemistry of living systems and the exciting discoveries on the molecular nature of enzyme action have paved the way for recognizing the importance of enzymes.[13]



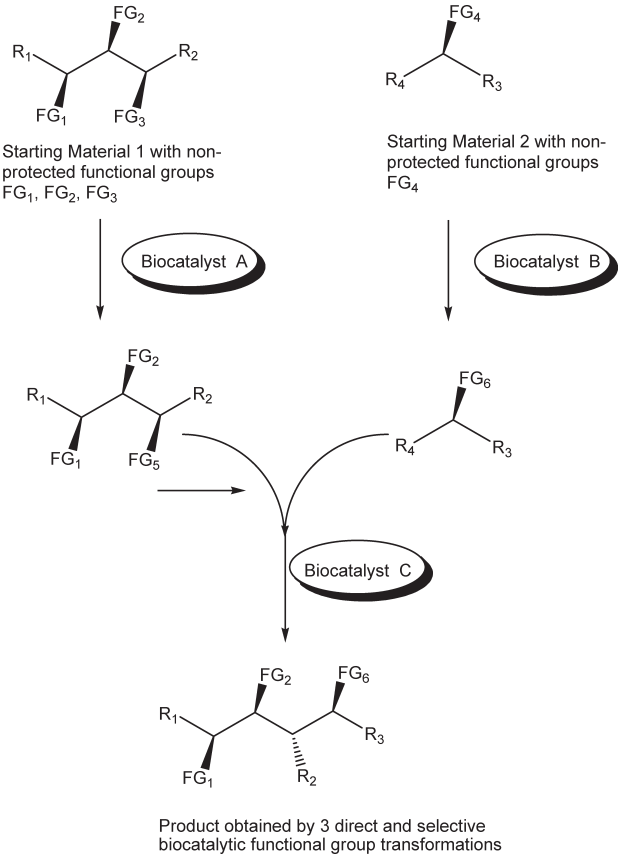
F i g . 2 – *Resolution of racemic tartaric acid by Louis Pasteur 1858*

Scientific and technological progress in the molecular and engineering sciences and their integration towards viable industrial processes has created sustainable value by enabling a large range of industrial biotransformations.[14–16] Therefore, reaction development of selected examples will be discussed in the following sections first from a molecular perspective, then from an engineering perspective, and subsequently from the practical overall perspective.

Molecular perspectives

The creation of value by converting abundant and easily available raw materials into products of use for the quality of life continues to be a common goal of both nature and human societies. The micro- and macroeconomic value creation by natural biotransformations of raw materials on our planet has been central to life and the knowledge of the molecular diversity of natural compounds is far from complete. The molecular economy of biocatalytic transformations in nature has its counterpart in the micro- and macroeconomic aspects of chemical syntheses in industry, where raw material costs, labour, energy and waste costs are related to the atom-, step- and redox-economy of the overall process. An area where these aspects have been particularly pronounced, is the manufacturing of asymmetric molecular architecture. The fascinating asymmetry of living organisms like snails[17] or the importance of chiral molecules in the chemical and pharmaceutical industry,[18] justify the attention given to clean manufacturing methods for products with high stereochemical purity. The three basic approaches discovered already in the 19th century by Louis Pasteur, involve the separation of enantiomers from a racemic mixture, asymmetric synthesis by one or more stoichiometric and/or catalytic steps and the conversion of chiral compounds from renewable natural raw materials. The standardization of chirality description by the Cahn-Ingold-Prelog rules[19] and the demonstration of the enantioselective catalytic action of enzymes by John Cornforth[20] stimulated research into the use of enzymes for asymmetric synthesis. The inherent chirality of enzymes due to the chirality of its amino acid constituents has been applied in an ever-increasing number of reactions for preparing molecular asymmetry with high efficiency, enantioselectivity and yield.[7–10] The applications of different classes of enzymes like oxidoreductases, transferases, hydrolases and lyases have been growing along various directions of synthesizing enantiomerically pure products by resolutions of racemic mixtures, asymmetric synthesis or bioconversions from chiral natural raw materials. The high efficiency and selectivity of enzymes in catalyzing functional group transformations is however not restricted to asymmetric transformations but has been also useful for many catalytic chemoselective conversions where no chiral center was involved. The molecular perspective towards organic and enzymatic reactions and the organic reaction types catalyzed by different classes of enzymes continue to be an inspiring and fruitful approach.[21–23]

Safety, health, energy and environment aspects of chemical production technologies have become key determinants of synthetic routes and are very much related to the reaction design. With the increasing number of enzymes becoming available, not only can biocatalytic tools and methodologies provide important improvements in these aspects, but can also reduce the amount of waste.[24] Even



F i g . 3 – *Sustainable Functional Group Conversions from starting material to product*

more decisive reasons for the choice of a particular biocatalytic reaction is the non-availability of the corresponding organic reaction type, avoiding harsh reaction conditions, which would lead to side reactions, or the selective and protecting-group-free approach schematically illustrated in Fig. 3.

Biocatalytic oxidations combine the utilization of mild oxidants like molecular oxygen, environmentally friendly solvents like water and highly selective and orthogonal non-stoichiometric oxidation reactions in an ideal way with the advantages of natural or recombinant oxidizing biocatalysts.[25,26] This is an attractive alternative to classical chemical oxidation reactions, where the simultaneous presence of reactive oxidants, flammable organic solvents and toxic auxiliary reagents requires special safety, health and environment precautions. The prime importance of biocatalytic oxidations goes however far beyond improvements in safety, health and environment issues. Biocatalytic oxidation can follow asymmetric reaction paths with remarkable selectivity and versatility as well as improved compatibility with labile functional groups.[26] Since the early examples of the C5-oxidation of D-(-)-sorbitol to L-(-)-sorbose catalyzed by *Acetobacter suboxydans* in the synthesis of vitamin C[27] and the 11α-hydroxylation of progesterone catalyzed by *Rhizopus arrhizus* in the synthesis of steroid hormones,[28] the use of biocatalysts in oxidation reactions has been growing.[25,26]

The synthetic applications of oxidases are simple and therefore a variety of alcohols, amino acids, amines, carbohydrates, metabolites, nucleosides and other building blocks have been oxidized enantioselectively by suitable oxidases. Among the variety of enzymatic routes for the production of chiral amino acids from the racemic aminoacid substrates,[29] the use of D- and L-specific amino-acid oxidases has been widespread.[8] The peroxides, which are formed naturally as reaction byproducts of the enzymatic oxidation of amino acids and often negatively influence enzyme stability, can be easily removed selectively by enzymatic oxidation with catalase. The resolution of racemic m-DL-tyrosine by immobilized D-aminoacid oxidase from *Trigonopsis variabilis* and a subsequent Pictet-Spengler reaction has given rapid access to enantiopure (S)-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline.[30] The yield can be further enhanced by non-enantiospecific chemical reduction of the oxidation product.[31]

With oxygen being available as abundant natural oxidant and electron acceptor, the reactions, where one or both of the two oxygen atoms of molecular oxygen are introduced selectively into an organic substrate, are of great synthetic interest. One-oxygen insertion reactions into organic substrates like hydroxylations, epoxidations, Baeyer-Villiger oxidations, heteroatom oxidations have catalyzed a variety of monooxygenases, with the other oxygen atom ending up in a molecule of water.

The Baeyer-Villiger reaction has been a key synthetic transformation for more than a century. Since the selective oxidation of one out of several ketone functions in an enantioselective way is a competence of biocatalytic systems, asymmetric Baeyer-Villiger oxidations and asymmetric heteroatom oxidations are best catalyzed by biocatalysts. Baeyer-Villiger monooxygenases have been a very useful group of enzymes due to their highly enantioselective oxidation of linear ketones to esters, cyclic ketones to lactones, N-and S-heteroatom oxidations.[32,33] The increasing number of recombinant Baeyer-Villiger monooxygenases, explorations and systematic extensions of the substrate range of these enzymes as well as progress in the biocatalytic process design has significantly broadened the range of applications for enzymatic Baeyer-Villiger oxidations in organic synthesis.[34–38]

The selective introduction of two atoms of molecular oxygen into an organic substrate can be achieved by a number of different reactions.

Sharpless asymmetric cis-vicinal dihydroxylation has rapidly become a most important reaction in organic chemistry. Dioxygenases as biocatalysts represent an interesting alternative[26] and have been used for the preparation of more than 300 cis-vicinal diols.[39] The tolerance of dioxygenases like the recombinant toluene dioxygenase and chlorobenzene dioxygenase to other functionalities like the nitrile group[40] is of interest for the synthesis of nitriledihydrodiols and their corresponding carboxylic acids.[41]

The early investigations on the product stereochemistry of D-sorbitol oxidation using microbes by Tadeus Reichstein[27] and ketone reduction using dehydrogenases by Vladimir Prelog[42] have inspired the still ongoing development of numerous biocatalytic reactions. Dehydrogenases and ketoreductases have therefore become excellent tools for synthesizing chiral alcohols,[43] hydroxy aldehydes,[44,45] hydroxy acids and amino acids, both in the oxidation and the reduction direction.[46,47]

The simple availability, preparation and application of hydrolases like acylases, proteases, esterases and lipases has been instrumental in the early introduction of enzymatic methods in organic chemistry. The growing number of applications for pig liver hydrolases as practical biocatalyst for asymmetric hydrolysis reactions in more than 100 years[48,49] are an illustration of the straightforward integration of this biocatalytic methodology into standard chemical synthesis. In addition to hydrolytic kinetic resolutions of racemic esters, hydrolases have been excellent chiral catalysts for enantioselective desymmetrization of meso- and prochiral esters by elimination of symmetry elements precluding chirality.[50] Acids, alcohols, amines, lactones, amino acids and other chiral building blocks are among the products most often prepared.

Epoxide hydrolases[51–53] have been used for the preparation of chiral diols and epoxides[54,55] by hydrolytic kinetic resolution of racemic epoxides or by enantioselective desymmetrization of meso-epoxides.

Nitrilases have attracted considerable synthetic interest for the direct, mild and selective conversion of nitrile functional groups to carboxylic acids,[56] because nitrilase-catalyzed reactions avoid the usual harsh chemical reaction conditions of strong acids and bases at high temperature and therefore preserve labile functional groups. Nitrilases have also been used for the synthesis of chiral carboxylic acids and nitriles by hydrolytic kinetic resolution of racemic nitriles[57] or by enantioselective desymmetrization of prochiral dinitriles.[58]

The increasing structural and functional characterization of transferases has also raised the interest in their utilization for a diverse range of synthetic reactions. Transfer reactions of the hydroxyacetyl-group from a variety of ketol donors to a broad range of (2R)-hydroxyaldehydes can be catalyzed efficiently by transketolases with full stereocontrol and high conversion yields. Transketolase-catalyzed two-carbon chain extensions using the irreversible ketol donor β-hydroxypyruvate have been important for the stereoselective synthesis of 2-keto-(3S,4R)-diols.[59–62]

One area where selective biocatalytic transfer reactions have a tremendous influence on the E factor is the synthesis of carbohydrates and glycocojugates, because classical chemical synthesis using protection-deprotection schemes for each glycosidic bond generates waste in stoichiometric amounts. The large-scale preparation of highly specific natural and recombinant glycosyltransferases for synthetic applications and the corresponding NDP-sugar donors have been instrumental for establishing highly efficient biocatalytic glycosylations. Rapid and complete regio- and stereoselective glycosylations can be achieved in water without the detour of protecting other functional groups and solving solubility problems.[63,64]

Transfer reactions involving the formal transfer of the amino group from a donor to a ketone or aldehyde acceptor have been known for more than 60 years and the excellent selectivity of recombinant aminotransferases is finding practical applications for the synthesis of both chiral and non-chiral amines.[65,66]

The admirable molecular diversity of nature's diverse donors to be transferred to various acceptors in reactions catalyzed by transferases as the ones mentioned above and others like carboxylases, methyl- and prenyltransferases, sulfo- and phosphotransferases is of both fundamental and practical interest.

The use of lyases in the formation of new carbon-carbon bonds for constructing complex structures from simple building blocks is promising, because their atom and step economy can be improved. Aldolase-catalyzed synthesis of lactols and N-polyhydroxylated compounds and their subsequent conversion to the correspondding products has been performed in a highly diastereoselective manner.[67–70]

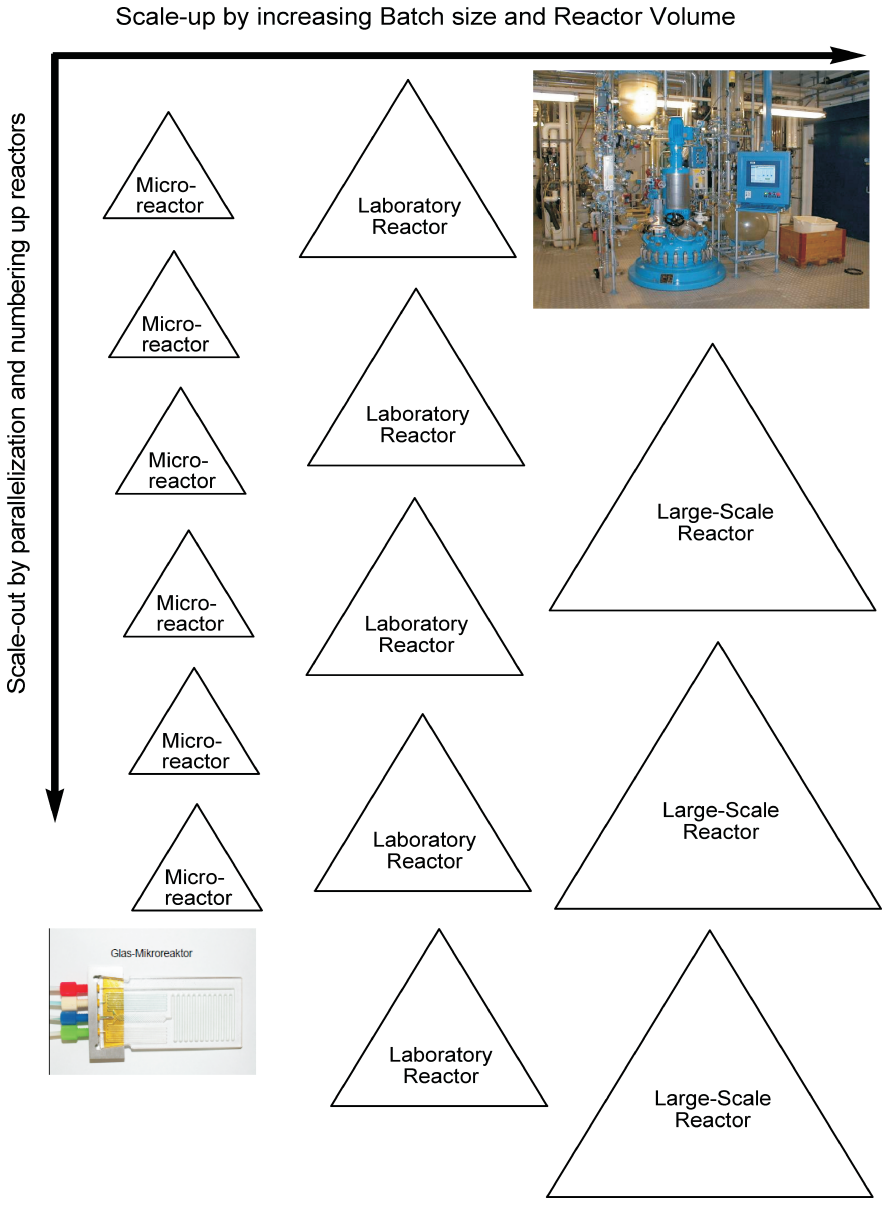
Molecules of increasing complexity can be built by multi-step enzyme conversions in one-pot or by multiple enzymes expressed in one cell. This is of practical industrial interest in the synthesis of compounds, where the chemical total synthesis is not competitive.[71] Robust processes to obtain the desired complex compound require however the de-

velopment of advanced analytical in-process controls.[72]

Shifting the molecular perspective from the reaction type to the product types, biocatalysts have not only been applied for the synthesis of small molecular weight compounds, but also as catalysts for polycondensations, ring-opening polymerizations, oxidative polymerizations and polymer modifications.[73] Polyphenols, polyanilines and vinyl polymers have been prepared by oxidoreductase-catalyzed oxidative polymerization, while polysaccharides, cyclic oligosaccharides and polyesters have been obtained by transferase-catalyzed polymer synthesis. Hydrolase-catalyzed polycondensations or ring-opening polymerizations have been used for the preparation of polyesters, polycarbonates, polyamides, polyphosphates and polythioesters.

Engineering perspectives

The value creation process is not complete with the discovery of a new bioconversion from a starting material to the product. The development of a suitable industrial process involves upstream technologies, the actual bioconversion and downstream processing like product recovery and purification, all of which can have a significant influence on the final design of the overall process. Process analysis, process design and improvement of equipment, processes and their operation aiming at process intensification play a key role for manufacturing products at industrial scale. Sustainable process engineering for the production of larger amounts of product can be achieved in different ways, as shown in Fig. 4, either by engineering larger reac-



F i g . 4 – *Sustainable Process Design from starting material to product*

tors and equipment or by parallelization of the same reactor and equipment size. Reduced material requirements and increased speed of process development for classical reaction scale-up through reactors and equipment of increasing size can be achieved by microscale techniques using microwell-plates, microbioreactors or microreactors.[74–76] The application of microreactor technology to biocatalytic processes extends to synthesis[77] and is of much interest for cases where aqueous and organic two-phase systems require efficient mixing and toxic reagents as e.g. in the hydroxynitrile lyase-catalyzed synthesis of cyanohydrins in microreactors from the corresponding aldehydes and in-situ generated HCN.[78]

A prerequisite is the scale-up of the production processes for the starting material as well as for the biocatalyst. While the starting material is not considered here, the scale-up of the biocatalyst production already requires a key decision concerning the form of the biocatalyst to be used (isolated enzyme, immobilized enzyme, whole cell). Other key decisions include the reactor type, reaction medium, reaction conditions, the sequence and timing of process steps, technology and equipment for downstream processing and purification. The identification of reaction and downstream processing bottlenecks to be overcome has been essential for the development of large-scale processes.

The scale-up of the biocatalytic Baeyer-Villiger oxidation of racemic bicycloheptenone to the two regioisomeric lactones (-)-(1*R*,5*S*)-3-oxa-bicyclo [3.3.0]oct-6-en-2-one and (-)-(1*S*,5*R*)-2-oxabicyclo-[3.3.0]-oct-6-en-3-one, the corresponding down-stream processing and product purification illustrate the importance of the engineering aspects and their consideration in the early phases of the process design.[79–84]

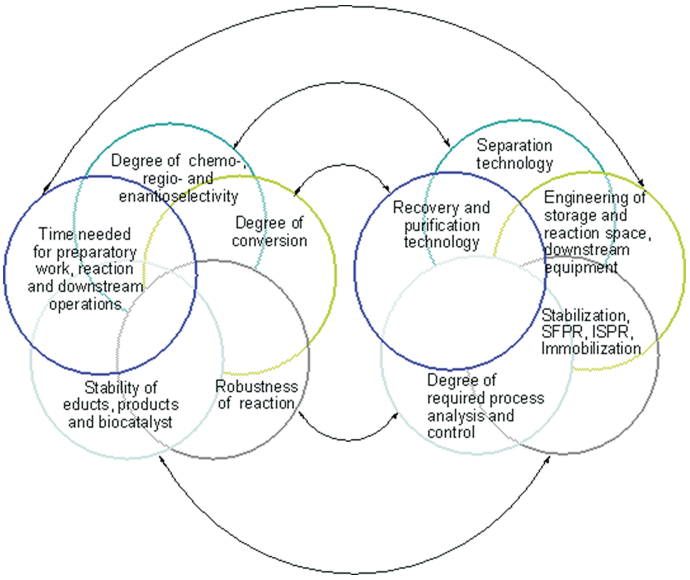
While whole cells were chosen as the form of the biocatalyst, microbial cell growth, enzyme induction and biotransformation have been devised as three separate parts, thereby allowing independent parameter optimization. The bottleneck of substrate and product inhibition for the Baeyer-Villiger Monooxygenase has been overcome by the process design of a resin-based substrate feed and product recovery process (SFPR)[79,81,82] or by direct substrate feeding below the inhibitory concentration and solvent extraction of the product.[80] The choice of the reactor type and specification is another key decision for which experimental data on the process and its parameters like the required amounts and concentrations of biocatalyst and oxygen and the degree of mixing are useful.[81] Technical improvements for oxygen mass transfer have been developed using sinter-metal spargers, which have been experimentally tested. The decision for the stirred tank reactor has been guided by the required oxygen and substrate supply.[83] The choice of the reaction medium depends on the solvent compatibility of the biocatalyst and the influence on the downstream and purification process steps. The substrate and product inhibition has been overcome by different engineering designs. The direct liquid-liquid extraction of the product from a whole-cell catalysed Baeyer-Villiger oxidation with controlled substrate addition can lead to significant differences in phase separation times depending on the utilized reaction medium. In the SFPR process design, the lactone product is obtained after adsorber separation from the reaction mixture and a washing step.[83] The downstream processing and the purification of the two chiral regioisomeric lactones, obtained as products in a 1:1 mixture in nearly enantiopure form (ee > 98 %) and good yield, has been a major bottleneck. Simulated Moving Bed Chromatography has been established as robust large-scale purification technology achieving a tremendous reduction in solvent consumption.[84]

The decision whether to use whole cell biocatalysts or isolated functionally pure enzymes depends on various parameters like the location of the enzyme in the cell, the substrate-product transport abilities of the cell, possible interfering side reactions by other enzymes of the whole cells and the type of biocatalyst or biocatalytic reaction. Process simplification is therefore another major process design goal in order to allow the use of existing industrial large-scale equipment and process control.[85,86] The simple reactor configurations, used in large-scale chemical synthesis, have been a major factor for the success of hydrolase-catalyzed processes at large scale.[14–16,87] In case of the isolated enzymes operational stability, mass transport, separation and cost issues are important for reaction engineering. The retention of enzymes within the reaction space by membranes has been well established in the operation of fed-batch or continuous biocatalytic processes at a production scale of several hundred tons per year.[88] Production processes using immobilized enzymes[89] are also well established and the most adequate immobilization method can be selected from a variety of options.[90]

An ideal product recovery scheme would be based on an ideal bioprocess with complete conversion to a single product, so that no product-starting material or product-side product separation would be needed. Real product recoveries require however a versatile toolbox of downstream technologies to meet the demand for high-yield processes with minimum amount of work, energy and number of steps.

Value creation by integrated processes

The number of reaction steps, as well as product recovery and purification steps in-between, together with the yield, raw material and labour costs, is among the main factors determining the economics of an overall synthesis. It is therefore clear that the replacement of multiple downstream processing steps or multiple reaction steps by single-stage processes can improve operational efficiency and cost issues. Integration of process steps can also be done between a reaction and a downstream processing step, as Louis Pasteur has already demonstrated in the biocatalytic tartaric acid resolution process.[11,12] In a one-step reaction, the integration should not add new problems but simplify the overall process and make it better scalable and more robust so that small deviations do not lead to a failed reaction. For a two or multi-step reaction, a sequence of reactions steps would ideally be integrated into a single step. If this were not possible, one option would be to make the optimum conditions for each of the reactions compatible with each other so that the overall reaction could be performed in one pot. In many cases however, the biocatalytic reaction step comes before or after a series of chemical reaction steps, which have to be worked through one after another. Even then, a minimum of integration is required and the choice of the right interfaces, as e.g. information storage, reaction medium, protecting group chemistry and purification/separation processes, between biocatalytic and classical organic synthetic reaction steps are important. In case of incompatible reaction conditions, new enzymes with the desired properties and activities may be engineered or screened from nature. The goal of shifting thermodynamic equilibria to the product side can on the other hand justify the integration of an additional irreversible reaction step or an in-situ product removal/separation step. In any case, the value creation in the overall process depends on various interactions between molecular and engineering aspects as shown in Fig. 5 and the further exploration of this exciting area looks promising.



F i g . 5 – *The interconnection of molecular and engineering aspects in the overall process*

Outlook

Biocatalysts, as nature's privileged catalysts for achieving high-performance transformations in living biological systems with the resources and energy available, are a good choice for developing selective chemical reactions with similar high molecular economy and efficiency without negative effects on biological systems. This is of industrial interest not only because of cost savings, but also because of safety, health, waste minimization, resource and energy efficiency. The requirement for selective transformations and the reduction in the extensive use of protective groups during the total synthesis of multifunctional molecules is clearly favourable for biocatalytic reactions.

The steady progress in building biocatalytic single-step reaction platforms, which are modular and scalable,[24] enables the evaluation of the compatibility with the development of chemical reaction steps.[91] Although thousands of new biocatalysts have been discovered and characterized over the last 50 years, the continued search and development of novel enzyme functions is key for new or improved synthetic methodologies,[92] especially where direct organic reactions are difficult or unknown. The tremendous progress in genomics and proteomics with the rapid sequencing technology are providing access to a huge amount of structural information, while the functional assignments have not kept up the same pace. The further development of robust, reliable and meaningful functional enzyme assays and analytical technologies for biocatalytic reactions is therefore essential for the discovery and optimization of novel biocatalysts or proteins with unknown functions.[93] It can be expected that the value of standardized functional information and EC numbers for novel enzymes, standardized reporting of biocatalytic reactions and rapid access will facilitate advanced studies building on these standardizations.[94]

It is worthwhile for the synthesis of small molecules to perform the comparison of different methods like total chemical synthesis, chemoenzymatic and enzymatic synthesis and engineering microbial cell factories on each individual target. Nevertheless, some general issues concerning safety, health,

economic and environmental sustainability and global boundary conditions of supply and demand, costs and availability of raw materials, labor and energy indicate the benefits of an increased application of biocatalysis in organic chemistry. While total chemical synthesis is reaching out to prepare compounds of ever increasing complexity, in the required amounts not yet available from nature, chemoenzymatic and enzymatic synthesis will continue its growth in new enzyme reactions as well as in the established reaction platforms for green large-scale manufacturing. The biological synthesis of valuable small molecules is focusing more on the construction and optimization of enzymatic pathways by metabolic engineering and synthetic biology.[95–101] The molecular aspects of the science of synthesis will benefit from interactions between the areas of inorganic, organic and biocatalysis. The engineering aspects of intensifying processes, increasing space-time yields, scaling issues and product recovery will also benefit from interactions between chemical and biochemical engineering. Progress in these molecular and engineering areas will influence the way of industrial manufacturing. Extending this perspective also to the excellent synthetic performance of biological systems and the global biosphere, the new knowledge gained at the interface of biocatalysis and organic chemistry will not only be useful for preparative purposes but also for areas dealing with complex systems like systems biology.

Abbreviations

CIP rules – Cahn-Ingold-Prelog rules for specifying molecular chirality

E Factor – Environmental Factor defined as mass ratio of waste to desired product

EC – Enzyme Commission

ISPR – in-situ product recovery

SFPR – in-situ substrate feed and product recovery

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