

Biocatalytic Process Design and Reaction Engineering*



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Review

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Biocatalytic processes occurring in nature provide a wealth of inspiration for manufacturing processes with high molecular economy. The molecular and engineering aspects of bioprocesses converting available raw materials into valuable products are therefore of much industrial interest. Modular reaction platforms and straightforward working paths, from the fundamental understanding of biocatalytic systems in nature to the design and reaction engineering of novel biocatalytic processes, have been important for shortening development times. Building on broadly applicable reaction platforms and tools for designing biocatalytic processes and their reaction engineering are key success factors. Process integration and intensification aspects are illustrated with biocatalytic processes to numerous small-molecular weight compounds, which have been prepared by novel and highly selective routes, for applications in the life sciences and biomedical sciences.

Key words:

molecular economy, retrosynthetic analysis, route selection, biocatalytic asymmetric synthesis, biocatalysts, biocatalytic process assembly, biocatalytic process prototyping, reaction engineering, process intensification, product recovery

Introduction

New functional molecular entities and synthetic methodologies, selectivity, resource efficiency and sustainability have been key performance drivers in the design of processes for manufacturing the desired products from adequate starting materials^{1–3}. While product purity and yield are in the forefront, resource efficiency and sustainability goals need to be met as well, as usually a number of auxiliary reagents and solvents are involved for each reaction and purification step, leading at the end to the accumulation of varying amounts of waste per unit of product manufactured^{4–6}. This is measured by the E factor, which has obtained a lot of attention in basic and industrial process design for assessing the minimization of waste and environmental impact of manufacturing processes over the past 25 years⁷. Waste minimization can be achieved by avoiding the use of auxiliary reagents in stoichiometric quantities, by highly selective reactions which do not lead to side products or follow-up products, and by high degrees of conversion which minimize purification media, auxiliary reagents, and solvent usage in product recovery. Nature provides a blueprint for process design by achieving the enormous tasks

of complex compound syntheses with high space-, time and stereocontrol. This is due to the great and growing diversity of natural, modified and designed biocatalysts, which have been described, and provide a tremendous knowledge base of renewable and non-toxic catalysts for resource-efficient biotransformations⁸. Biocatalysts are also versatile with respect to solvents as organic synthetic reactions in biological cells can be achieved in aqueous or membrane environments and no organic solvents are needed, thereby putting biocatalysis in an excellent position for a paradigm change of solvent use in organic synthesis⁹. Biocatalysis is therefore ideally suited for applying the concept of molecular economy (Figure 1) to the design of manufacturing processes and the development of a sustainable chemistry¹⁰. As the resource efficiency goal has been approached in parallel from different perspectives, interfacing chemistry with the biosciences¹¹ as well as bridging the molecular with the engineering sciences¹² is instrumental for successful industrial implementation¹³.

Biocatalytic process design can be inspired from the two extremes of exclusive chemical and biological methodologies. Bottlenecks and limitations in purely chemical manufacturing processes, the needs for selective new tools in total synthesis/diverted total synthesis and disruptive experiences to the quest that any molecular structure, no matter how complicated, can be constructed by the excel-

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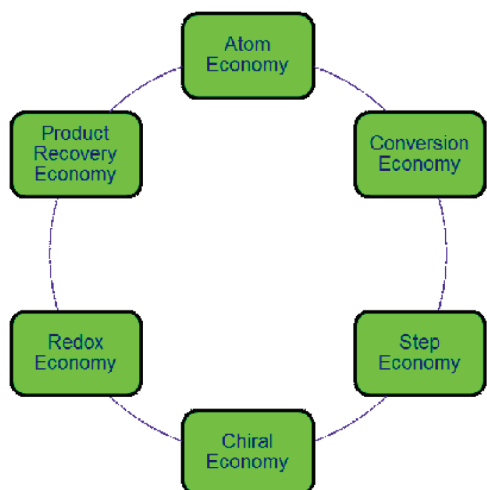


Fig. 1 – Manufacturing performance, selectivity, sustainability by molecular economy

lent tools of organic chemistry, can start the search for enzymes capable of catalyzing a particular reaction not possible with present synthetic methodologies. Metabolism, inhibition, regulation, and transport in purely biological manufacturing processes, arising from the perspective of why you should synthesize a compound yourself if a bug can do for it for you, can, on the other hand, require to rely on robust chemical reactions or to develop new synthetic methods for manufacturing.

From the organic chemistry side, great progress has been achieved in the area of green and sustainable chemistry with a broad range of highly selective and tailor-made biocatalytic transformation types, which are superior to the corresponding tools available, developed for an increasing number of substrates^{14–15}. As many biocatalytic reactions have moved successfully from laboratory to industrial scale^{16–19}, the interest to consider a biocatalytic reaction step already from the beginning in the design of a chemical manufacturing process as an option for a reaction in a synthetic sequence has been growing^{20–22}. From the other extreme of synthesizing a compound by biosynthesis in the fermentation of whole cells, the success of white and industrial biotechnology is due to the tremendous progress in biochemical and metabolic engineering, molecular biology and synthetic biology, which opened options for the design of complete biocatalytic pathways from simple starting materials to complex products^{23–25}.

Whether the manufacturing route originates from the chemical or the biological methodologies, a number of criteria which are considered in designing the route, are common to both, e.g. the design direction of old routes, with the advantage of building on established technologies or with the option to revitalize it, versus the design of completely new

routes with the aim of faster and shorter routes to the target compounds. The route architecture with its related use of reaction space and reaction time like a linear or convergent route, multi-step- or multi-component reaction, offers numerous opportunities for route design¹⁰. A great advantage of using biocatalysts for performing reaction steps in these routes are their privileged properties, like their chemoselectivity in transforming non-protected substrates and their enantioselectivity due to their inherent chirality. This latter property has been successfully used in biocatalytic reactions for resolving racemic mixtures to pure enantiomers, for desymmetrizing prochiral or symmetric substrates, and for catalytic asymmetric synthesis^{26–28}. The design of synthetic routes is not restricted to finding ways how to synthesize a product target from suitable starting materials, but can also be oriented towards an abundant and inexpensive starting material. If this starting material is renewable by nature, constantly accumulated as a side product or as waste in a large-scale manufacturing process, and no further use of these resources than disposal is envisioned, designing new processes improves resource efficiency. It is of increased interest for a number of industrial applications to shift the starting point of manufacturing routes from fossil-based to bio-based raw materials²⁹. Whether routes are oriented towards product targets, starting materials, functions, or diversity, biocatalytic process design is a key enabling framework.

Analysis and design tools

Routes oriented towards starting materials require a forward-looking analysis and the imagination of valuable target products, which could be made accessible from the given starting material, as shown in Figure 2 for the case of glycerol^{30–31}. Target product-oriented routes are routinely designed by a retrosynthetic analysis and a decision from which material to start with, as schematically shown in Figure 3. The integration of biocatalysis into classical retrosynthetic analysis is thereby important³² and demonstrated by the cases of the target products enantiomerically pure D- and L-lactaldehydes as well as KDG^{33–34}. Another key prerequisite for successful biocatalytic processes is the availability of adequate and reliable methodologies for bioprocess analysis at the start of development for the unambiguous identification and purity determination of the product. If analytical methods like enzyme assays for a particular bioprocess have not been described previously, their development is instrumental for bioprocess screening and development³⁵. Straightforward analytical methodologies for separating products from starting materials and

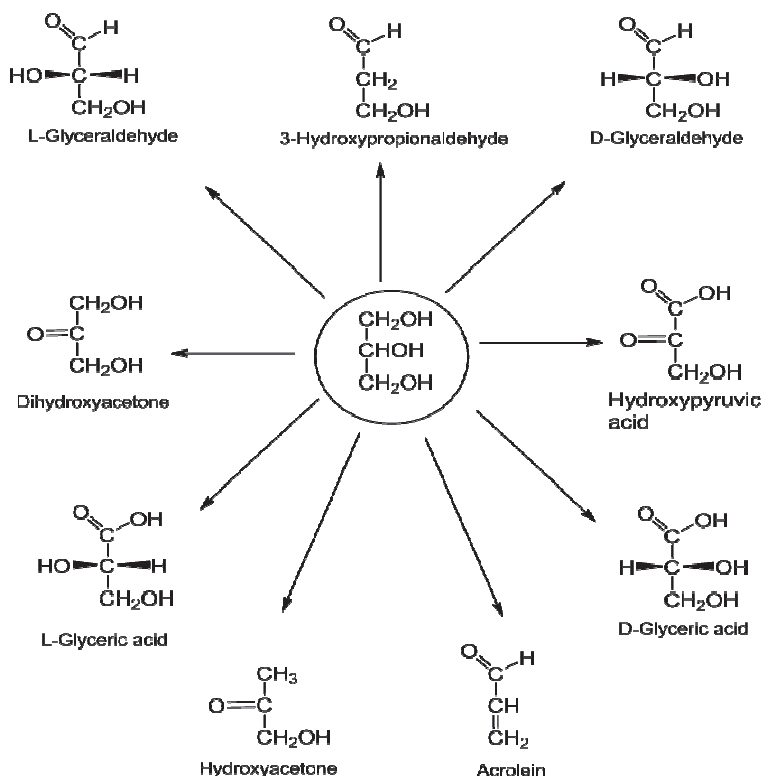


Fig. 2 – Starting material-oriented routes to products originating from glycerol

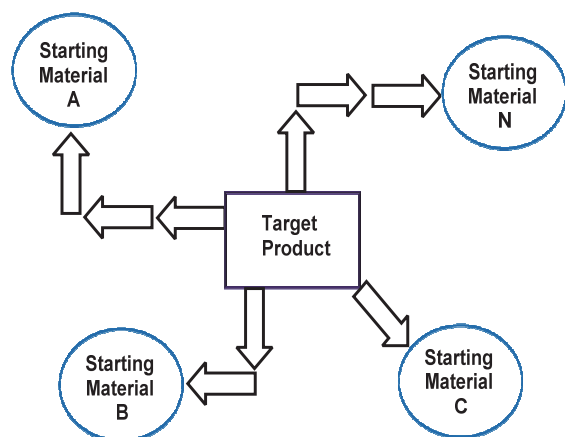


Fig. 3 – Target product-oriented routes

detecting product formation are not only valuable for development, but also for monitoring manufacturing processes. Industrial manufacturing processes demanding consistent batch-to-batch quality within the specifications are ideally developed the first time right with adequate analytical tools in order to achieve the desired quality by designing it from the beginning with the QbD methodology. This can be achieved by taking discrete samples during the manufacturing process, which are transported to the analytical instruments and analysed off-line or by designing on-line analytical instru-

mentation which are integrated into the manufacturing process as analytical in-process controls. PAT is of much interest for a better understanding of the process in development³⁶ or for controlling critical unit operations like reactions, workup, crystallization and drying³⁷. In the innovative transaminase-catalysed process for manufacturing the sitagliptin API³⁸, the particle size distribution of the API is an important quality specification. As the API particle size is dependent on the seeding point temperature of its crystallization from solution, which itself depends on the solution composition of each batch before crystallization, the analysis of the composition of the solution is important. This has been achieved by NIR spectroscopy of the sitagliptin free base, water, isopropylamine, DMSO and isopropylacetate concentrations in real time prior to the seeding point of sitagliptin crystallization³⁹. These real-time NIR measurements not only increased productivity and flexibility, but also enabled the implementation of a control strategy³⁹.

Biocatalytic process assembly and prototyping

Biocatalytic reaction platforms with numerous biocatalysts and their applications, established over the years for many reaction classes like reductions^{40–41}, oxidations^{42–44}, hydrolysis^{45–46}, aminations^{47–48} or phosphorylations^{49–50}, have paved the way for extending the biocatalyst applications to new substrates and for putting together new synthetic routes. Best practices in the assembly of synthetic biocatalytic as well as chemical synthetic reactions involve rapid prototyping of the most critical reaction steps in order to obtain the first proof-of-principle. It is thereby useful to overcome bottlenecks for single biocatalytic reaction steps, as shown in Figure 4 for selected examples, before proceeding to prototyping the whole sequence. The examples illustrate challenges in biocatalytic process development and different approaches how to tackle them. The synthesis of the pharmaceutical intermediate ethyl-(1R,2R)-2-(3,4-difluorophenyl)-cyclopropanecarboxylate to Ticagrelor has been achieved with 79 % yield and very high selectivity by engineering a truncated globin of *Bacillus subtilis* enzyme, which catalysed the cyclopropanation of 3,4-difluorostyrene with ethyl diazoacetate⁵¹. Among the many synthetic approaches to the neuraminidase inhibitor oseltamivir phosphate, the route including the enzymatic desymmetrization of a *meso*-1,3-cyclohexane-dicarboxylic acid diester as the

asymmetric versions and biocatalysts are not available, which is a disadvantage and needs to be overcome by interfacing biocatalysis with classical organic synthesis¹¹.

Reaction engineering

Reaction rate laws, targets and variable parameters for biocatalytic processes are different from chemocatalytic reactions due to effects of higher substrate and product concentrations, which are desirable to achieve high space-time yields, on the biocatalysts in industrial processes compared to regulated biocatalytic reactions in nature, different tools for shifting thermodynamic equilibria and solvent, temperature and pH influences on biocatalyst properties⁵⁷. Depending on previous knowledge of the biocatalytic reaction platform, the reaction engineering can work with mathematical optimization tools to define optimum reaction conditions for well-characterized reaction systems, with practical design-of-experiments methodology after prototyping or, if data are lacking, with experimental data acquisition on the characterization of biocatalytic reaction kinetics, biocatalysts, components and thermodynamics of the reaction system as a function of variable parameters⁴⁹. Unfavourable thermodynamics may be overcome by making the reaction

irreversible via the utilization of irreversible substrates, different reaction media or the coupling to subsequent irreversible reaction steps. In cases of stability mismatches between substrate or product stabilities and enzyme activities and stabilities, rapid progress can be obtained by optimizing process windows⁵⁸. Further optimization of reaction engineering parameters, such as the degree of conversion, selectivity, and specific productivity, require more work, but can lead to highly efficient synthetic procedures^{59–60}. In addition to high space-time yield, reaction engineering and process intensification aim at complete conversion to the final product in order to avoid laborious product isolation operations^{61–63}. Even with complete conversion, the final product also needs to be recovered and purified from the reaction system with high efficiency⁶⁴. In a systematic optimization of the biocatalytic reaction system using DoE parameter investigations for the manufacturing of the enantiomerically pure D- and L-lactaldehydes, the reaction engineering target values have been met and even surpassed with >99 % yield, >99 % ee at 250 g L⁻¹ substrate loading and 0.05 g L⁻¹ NADP cofactor requirement, after two phases of process development subsequent to screening³³. In the case of the biocatalytic resolution of (+)-cis/trans limonene oxide and (-)-cis/trans-limonene oxide, the choice of the most suitable reaction parameters, like substrate loading, pH, tem-

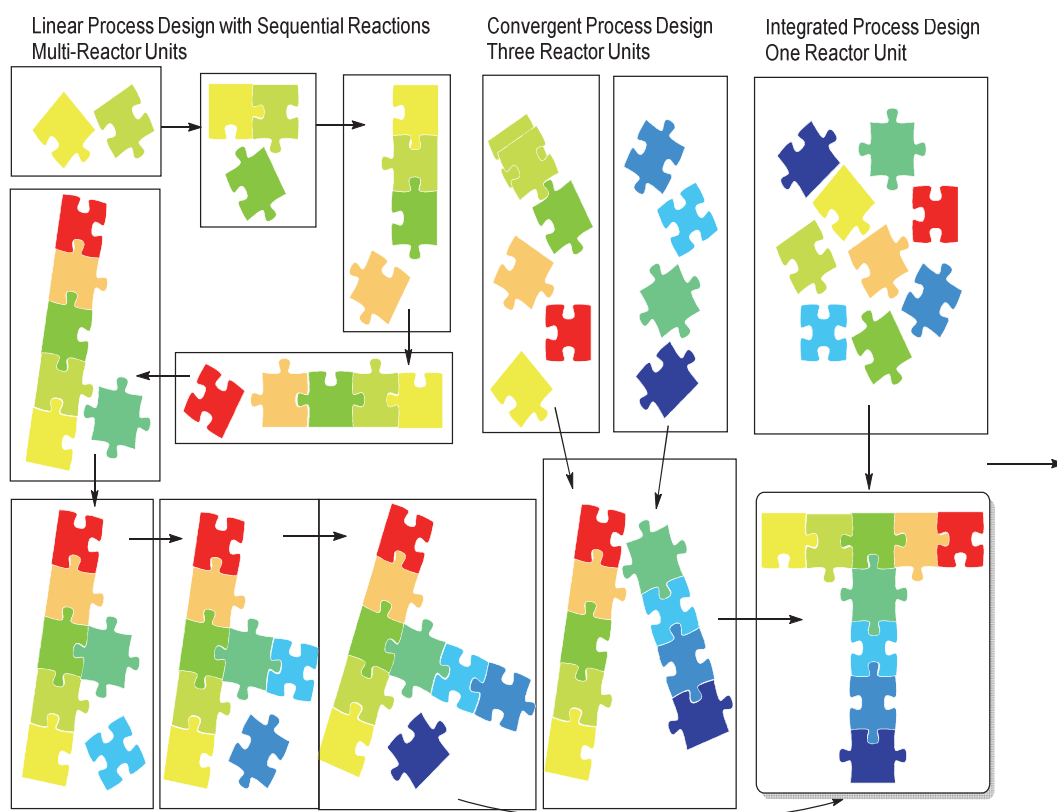


Fig. 5 – Bioinspired biocatalytic process design strategies

perature and cosolvents, was based on experimental investigations of limonene oxide stability, epoxide hydrolase stability and activity as a function of pH and temperature^{55–56}. The optimization of the reaction conditions for these epoxide hydrolase-catalysed resolutions has led to improved space-time yields and specific productivities with up to 2 M substrate concentrations and lower enzyme consumption numbers, without the need for cosolvents⁵⁶.

Outlook

The design of new biocatalytic routes to compounds which have not been accessible up to now or had to be manufactured over a multitude of reaction steps with low yields is an attractive and powerful option. With the rapid and versatile technologies of molecular biology and protein expression novel straightforward biocatalytic routes to such products can be designed by taking advantage of nature's established reaction types, such as biocatalytic phosphorylations, like the biocatalytic phosphorylation of L-arginine to N_ω-phospho-L-arginine⁶⁵ or the glycerate-2-kinase-catalyzed synthesis of D-glycerate 2-phosphate⁶⁶. This enables the extension of biocatalytic process design towards a) reactions with new substrates and enzymes within established biocatalytic reaction platforms, and b) the development of novel biocatalytic reaction platforms. Screening for suitable biocatalysts, substrates and reaction conditions represents significant research efforts, and automation of the workflow can accelerate the development⁶⁷. Microscale technologies offer thereby strategic advantages for the development and realization of biocatalytic processes and subsequent product recovery steps⁶⁸. Reaction engineering for biocatalytic processes in flow is flexible and simplified, since the developed processes can be parallelized or scaled out instead of the classical scale-up procedure in going from small-scale to large-scale manufacturing. In both batch and flow systems, biocatalytic process intensification and the construction of coupled reactions steps all together in one unit or sequentially in two or more units, depending on compatibilities, is of much interest. This biocatalytic process design (Figure 5) follows nature and is attractive, since purification of intermediates can be avoided. Thermodynamic limitations can be overcome by coupling unfavourable reactions with subsequent irreversible reactions. Finally, side reactions may be prevented as biocatalytic reactions are likely to be orthogonal, of course to be verified experimentally. The times are changing towards biocatalytic process design and reaction engineering.

Abbreviations

API	– Active Pharmaceutical Ingredient
DoE	– Design of Experiments
E	– Ratio of the waste generated per unit of product manufactured
EC	– Enzyme Commission
ee	– Enantiomeric excess
KDG	– 2-Keto-3-deoxy-D-gluconate
LC-MS	– High-Performance Liquid Chromatography with detection by Mass Spectrometry
NIR	– Near Infrared
PAT	– Process Analytical Technology
QbD	– Quality by Design

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