Review Article

BIOCATALYST : PRODUCTION ,CHARACTERISTICS AND FUTURE PROSPECT

*Hussein Salih Hussein[1], K. S. Kulkarni[2], S. N. Shinde [2]

Address for Correspondence

[1]Student, M.Tech. Chemical Engineering, [2]Associate Professor, Department of Chemical Engineering, Bharati Vidyapeeth Deemed University College Of Engineering , Pune-43.

ABSTRACT

The general features of biocatalyst that led to their widespread use in chemical engineering industry are highlighted as well as the details of their impact on selected processes. Biocatalyst offer the basis of many of these technological solutions provided efficient and balanced co operations between industry and academia are further developed.

INTRODUCTION

Over the last 20 years, many reservations with respect to biocatalysis have been voiced, contending that: (i) enzymes only feature limited substrate specificity; (ii) there is only limited availability of enzymes; (iii) only a limited number of enzymes exist; (iv) protein catalyst stability is limited; (v) enzyme reactions are saddled with limited space–time yield; and (vi) enzymes require complicated co substrates such as cofactors Biocatalysis is one of the main pillars of applied biotechnology, defined by the European Federation of Biotechnology as the "integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogs for products and services", and according to EuropaBio, 2003, "White Biotechnology is the application of Nature's toolset to industrial production". Both definitions have in common that biotechnology, and thus biocatalysis, are looked at as interdisciplinary sciences.

Production of Biocatalyst

Biocatalyst development requires, initially, identification of the required enzyme activity, in terms of the desired chemical reaction substrates and products, and thereafter, the identification of sources of such enzyme activity. Conventional microbiological approaches using selective screening may be adequate for this purpose, but the range of sophisticated modern molecular technologies now available has provided the biotransformations chemist with an immensely enhanced choice of potential enzyme sources. Exploration of extreme environments, and exploitation of genomes using advanced technologies such as total environmental DNA sampling, cloning technologies, and genetic manipulation, have altered the approach in biocatalysis to the point where rather than develop a purpose for an enzyme, we design and develop an enzyme for a purpose. Once a suitable source of the desired enzyme activity has been found, an efficient production system for the enzyme would be required, whether it is to be applied as an isolated enzyme or encapsulated in cells. In a typical conventional biocatalysis process, the cells would be grown in a fermentation system, then harvested and used as whole cells, or the enzymes would be extracted and then used in an isolated form, for the biocatalytic reaction. The fermentation would be optimized to produce maximum biomass, and the cells would be harvested when the desired enzyme activity was maximal. Further biocatalyst development would involve characterization of the enzyme production by the cells and the kinetics and substrate selectivity of the enzymes involved. Optimization of fermentation and biotransformation reaction conditions may involve extensive investigation and modification of the biocatalyst production system and the reactor conditions. For industrial application, further development would require stabilization of the biocatalyst so as to ensure consistent biocatalytic activity over prolonged reaction times, in terms of product fidelity as well as chemical productivity and biological stability. The ideal biotransformation system would include:

- an efficient enzyme production system
  - using a readily culturable microbial source
  - genetically stable, nonpathogenic strain
- an efficient biocatalyst
  - used as resting cells or purified enzymes
  - highly stereoselective
  - high activity in the desired reaction
  - flexible substrate selectivity
  - minimal side reactions
- a stable biocatalyst
  - stable under optimal reaction conditions
  - menable to immobilization/stabilization
  - amenable to bioreactor constraints

We have a number of choices, in terms of improving biocatalysts for industrial application, including metabolic engineering of the biological source (the cells), for instance, to alter the enzyme expression by the cells. Alternatively, genetic engineering might lead to, for instance, enhanced enzyme selectivity or elimination of side reactions, or production of recombinant organisms in which desirable characteristics of different enzymes from more than one organism can be combined. An alternative to molecular approaches is the reaction engineering approach, in which the bioreactor conditions are

manipulated to provide an environment suited to the biocatalyst and the biotransformation. Reaction engineering allows for the development of:

- continuous fermentation/biotransformation processes
- continuous addition of substrate and recovery of product
- efficient use of immobilized or stabilized enzymes/cells
- use of heterogeneous solvent systems (organic/aqueous)
- expanded substrate range

Generation and identification of novel biocatalysts

The generation of novel biocatalysts using genetic or biochemical methods, as well as the identification of extremophiles or other sources for enzymes with unusual properties, represent important objectives in the biotechnological development of biocatalysis. An example of the genetic approach is provided by the recent work of Moore and Arnold [16**], who report the 'directed evolution' of an esterase to be used for antibiotic deprotection in aqueous-organic solvents through sequential generations of random mutagenesis and screening. One esterase variant was found to perform as well in 30% dimethylformamide as the wild-type enzyme does in water, reflecting a 16-fold increase in esterase activity. The random pairwise gene ecombination of two positive variants led to a further twofold improvement in activity. Considering also the increased expression level achieved. during these experiments, the net result of four sequential generations of random mutagenesis and one recombination step was a SO-60-fold increase in total activity. The authors point out that although the contributions of individual effective amino acid substitutions to enhanced activity are small (<twofold increases), the accumulation of multiple mutations by directed evolution allows significant improvement of the biocatalyst for reactions on substrates and conditions not already optimized in nature. The positions of the effective amino acid substitutions were identified using an esterase structural mode1 based on homology to acetylcholinesterase and triacylglycerol lipase. None of the substituted amino acid residues appear to interact directly with the antibiotic substrate, which underscores the difficulty of predicting mutational effects in a 'rational' design effort. A more molecular approach to the generation of novel biocatalysts is illustrated by the work of Kim et a/. [17**] on the construction of hybrid restriction enzymes. Two novel site-specific endonucleases were successfully produced by linking two different zinc-finger proteins to the cleavage domain of F&I endonuclease. Both fusion proteins are active, and under optima1 conditions each cleaves DNA in a sequence-specific manner. Thus, these results demonstrate the feasibility of constructing a variety of artificial nucleases that will cut DNA near predetermined sites, by linking the FoAI endonuclease module to a specificity module such as a zinc-finger motif.

Characteristics of Biocatalysis as a Technology

1. Dtributing Disciplines and Areas of Application

From different disciplines, biotechnology and biocatalysis are seen from very different angles and perspectives (Figure 1). Chemistry and chemists emphasize a *molecularly-oriented* perspective dominated by compounds and transformations, whereas chemical engineering and thus chemical engineers favor a *process-oriented* perspective of reactions and processes; lastly, biology and its practitioners contribute a *systems-oriented* perspective of description at the organism level as well as in their view of evolution. Different parts of each of the three isciplines are needed for the successful practice of biocatalysis: biochemistry and organic chemistry from chemistry; molecular biology, enzymology, and protein (bio) chemistry from biology; and catalysis, transport phenomena, and reaction engineering from chemical engineering are indispensable. Both biotechnology and biocatalysis are interdisciplinary areas; as most practitioners tend to hail from one of the three major contributing disciplines, hardly anybody has an equally strong command of all the sub-disciplines of biocatalysis.

There are not only many contributing disciplines for biotechnology and biocatalysis, but also many *areas of application:*

- production and transformation of compounds, mainly in the chemical and pharmaceutical industry,
- analytics and diagnostics, mainly in medicine, and
- environmental protection and bioremediation (reconstruction of the environment).

The areas of application differ from the *industries* which apply them; the most important ones are the pharmaceuticals, food, fine chemicals, basic chemicals, pulp and paper, agriculture, medicine, energy production, and mining industries

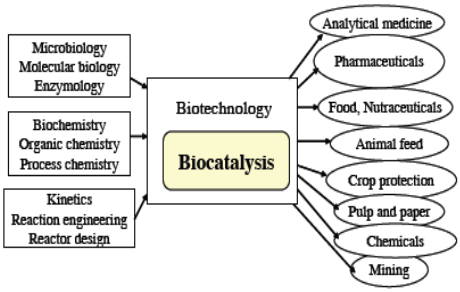

Figure 1 Central role of biocatalysis and biotechnology between interdisciplinary feeder sciences (biology, chemistry, chemical engineering science) and multiple user industries.

2. Characteristics of Biocatalytic Transformations

Biotechnological transformations include a broad range of processes, ordered according to the number of biologically performed process steps and the complexity of the substrates (Figure 2):

- *Fermentations* transform raw materials such as sugar, starch or methanol, often in industrial mixtures such as molasses or corn sleep liquor as carbon sources with living cells to more complex target products.
- *Precursor fermentations* start with defined educts and transform these, again with living cells, to the desired target products.
- *Biotransformations* transform defined precursors in a series of defined (not always known) steps with enzymes or resting cells to a desired target product.
- In *enzyme catalysis,* frequently *crude extracts* or *partially purified enzymes*, which only have to be free of side activities, are utilized for the transformation from defined substrate to target product.
- Purified enzymes are rarely used for the production of chemicals, possibly only for the production of highly priced fine chemicals such as pharmaceutical actives.

The limits between the areas are blurred: biotransformations and enzyme catalyses with crude extracts or pure enzymes are often summarized under the term

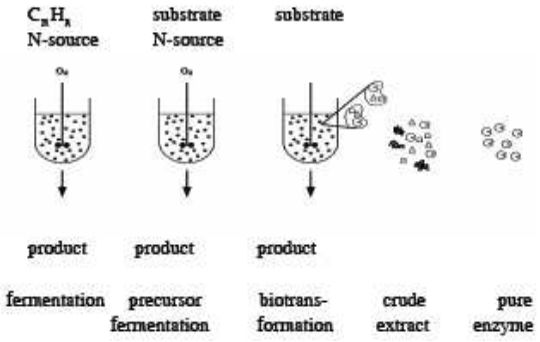

Figure 2 Biocatalysis as a continuum between fermentation and transformations with pure enzymes.

Future prospects

Innovative strategies for biocatalytic flavour generation will certainly take advantage of the immense progress currently being made in the emerging fields of functional genomics, proteomics, protein and metabolic engineering. As an increasing number of genome sequence data becomes available, the improved genomic and proteomic methodologies provide access to new enzymes including those playing key roles in plant flavour biosynthesis. Modern microbial host vector systems simplify the functional expression and molecular and biochemical characterization of heterologous enzymes. An increasing amount of research work is being published which gives insight into plant biosynthesis by 'isolation, cloning and expression' approaches. Croteau and co-workers elucidated the enzymatic catalysis and stereochemistry of (−)-limonene hydroxylations in different mint species by heterologous expression in *E. coli* and *S. cerevisiae* (Haudenschild *et al.* 2000, Wüst *et al.* 2001). In peppermint (*Mentha* x *piperita*) (−)-limonene is converted into (−)-*trans*isopiperitenol by a P450 monooxygenase regiospecifically oxyfunctionalizing the C3 position, the first reaction of a subsequent cascade of five enzymes leading to the important flavour molecule (−)-menthol. By functional expression in *E. coli*, a multifunctional *O*-methyltransferase, catalyzing the formation of 2,5-dimethyl-4-methoxy-3(2H)-furanone(DMMF), an impact compound in strawberry flavour, has successfully been characterized (Wein *et al.* 2002). These are only a few examples among many others to illustrate the progress currently being made by genetic engineering in flavour biosynthesis research. Functional expression of newly discovered, flavour-generating plant enzymes in bacteria or yeasts constitutes a potential starting point for a bioprocess-oriented optimization, but only the establishment of high-level expression systems can transfer them to real whole-cell biocatalysts. Additionally, molecular biological methodologiesare necessary to optimize biocatalyst properties, e.g. by conferring higher stereoselectivity or thermostability or even a different substrate spectrum. The active site of *Pseudomonas putida* heme monooxygenase P450cam, which naturally converts (+)-camphor to 5- *exo*-hydroxycamphor, was remodeled by site-directed mutagenesis and a double mutant showed completely different substrate and product spectra (Bell *et al.* 2001): (−)-limonene and *α*-pinene were hydroxylated with high regio- and stereoselectivities to (−)-*trans*isopiperitenol and (+)-*cis*-verbenol, respectively. A strategy of potential future impact on natural flavor production addresses the total synthesis of terpenoids by engineering the mevalonate-dependent isoprenoid pathway comprising eight genes from *S. cerevisiae* into *E. coli* (Martin *et al.* 2003). The goal is a bacterial cell factory providing the universal precursors of terpenoid flavour compounds, isopentenyl pyrophosphate and dimethylallyl pyrophosphate, by *de novo* synthesis. This might grant access to the variety of terpenoid flavours once the appropriate genes coding terpene synthases and functionalizing enzymes have been heterologously expressed in the same host strain. The

feasibility of novel biocatalytic flavour syntheses will not only depend on genetically improved biocatalysts but also on process engineering features. This is especially evident in the case of terpenoid flavour compounds, an area of high commercial interest to the industry due to the large number of keyaroma compounds which are in principle accessible by biotransformation of abundant natural terpene hydrocarbons (Schrader & Berger 2001). Biotransformation attempts have to overcome several drawbacks, such as the low water-solubility of the precursors, toxicity of precursors and products, and metabolic diversity which leads to unwanted by-products or further degradation of the target molecules. One example which shows that extremely high yields can nevertheless be obtained was given by Fontanille & Larroche (2003). Up to 400 g 2(*Z*)-methyl-5-isopropyl-2,5-hexadienal (isonovalal) l−1, an artificial fragrance compound for potential use in perfume formulations, was produced from *α*-pinene oxide within 2.5 h using 25 g precultivated biomass l−1 (*Pseudomonas rhodesiae* CIP 107491); the cells had been permeabilized by freezethawing and organic solvent treatment prior to use.

REFERENCES

1. Andreas S. Bommarius and Bettina R. Riebel ," Biocatalysis: fundamentals and applications " , Copyright © 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
2. Peter Grunwald , "Biochemical Fundamentals and Applications" , 2009, Imperial College Press
3. Stephanie G. Burton ,"Pure Appl. Che.", Vol. 73, No. 1, pp. 77–83, 2001.© 2001 IUPAC
4. J. Schrader, M.M.W. Etschmann, D. Sell, J.-M. Hilmer & J. Rabenhorst, Biotechnology Letters 26: 463–472, 2004. © 2004 Kluwer Academic Publishers. Printed in the Netherlands.
5. Morita T, Yoshida N, Karube I: A novel synthesis method for cyclodextrins from maltose in water-organic solvent systems. Appl Biochem Biotechnoll996, 56:31 l-324. Monsan P, Paul F: Enzymatic synthesis of oligosaccharides. FEMS Microbial Rev 1995, 16:187-l 92.
6. Kim YG, Cha J, Chandrasegaran S: Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Nat/ Acsd SC; USA 1996, 93:1156-l 160.