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Research review paper

Enzymatic and whole cell catalysis: Finding new strategies for old processes

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ABSTRACT

The use of enzymes and whole bacterial cells has allowed the production of a plethora of compounds that have been used for centuries in foods and beverages. However, only recently we have been able to master techniques that allow the design and development of new biocatalysts with high stability and productivity. Rational redesign and directed evolution have lead to engineered enzymes with new characteristics whilst the understanding of adaptation mechanisms in bacterial cells has allowed their use under new operational conditions. Bacteria able to thrive under the most extreme conditions have also provided new and extraordinary catalytic processes. In this review, the new tools available for the improvement of biocatalysts are presented and discussed.

Contents

1. Introduction

In the 1930s, the production by yeast of (*R*)-phenylacetylcarbinol, a precursor of the well known bronchodilator L-ephedrine, showed that a one-step transformation could be significantly important in organic synthesis (Hilderbrandt and Klavehn, 1930). It was also in the 1930s that the Polish Ernest A. Sym published several papers showing the action of porcine pancreatic lipase in organic media (Sym, 1930, 1936). However, his work was later forgotten because it appeared before the dogma that enzymes are active only in aqueous environments (Halling and Kvittingen, 1999).

Biocatalysis can be performed by both whole cells and isolated enzymes. The selectivity, including regio-, chemo-, diastereo- and enantioselectivity, and the mild conditions under which the biotransformations can be carried out, make this approach preferable to chemical reactions. In some cases, such as the selective hydroxylation of non-activated carbon atoms, biotransformations can even be the only known solution to produce a certain compound. In one-step reactions, isolated enzymes should provide significant benefit when compared to whole cells as no side-reactions should occur and substrates do not have to be transported across membranes. However, whole cells should present significant advantages in multi-step bioconversion processes such as the side-chain cleavage of β-sitosterol by *Mycobacterium* sp. NRRL B-3805 cells (Staebler et al., 2004).

Enzymes are proteins that are produced by living organisms to catalyse metabolic biochemical reactions necessary to all life processes. The enzymes are able to catalyse reactions at a rate far more rapid than it would occur without their action and under mild conditions, within a narrow range of pH and temperature. Furthermore, enzymes are able to continue to function under *in vitro* conditions and can catalyse reactions

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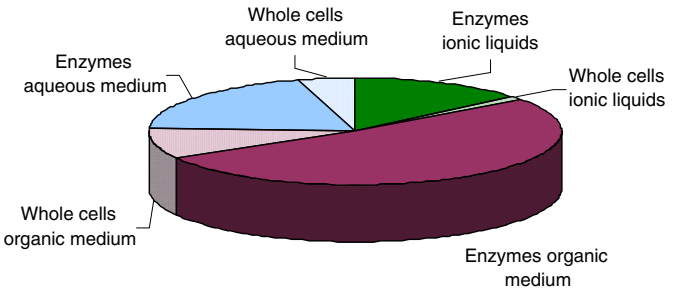

Fig. 1. Percentage of papers published with enzymes and whole cells in different reaction media.

in conditions not suitable for cell growth, although they only work efficiently under optimum pH and temperature conditions. In fact, the majority of papers published on enzymatic biocatalysis in the last decade, reported the use of enzymes in organic media and ionic liquids (Fig. 1). The use of pure enzymes in biocatalysis has several advantages such as the specificity for selected reactions, simple apparatus and procedures and better tolerance to co-solvents used to solubilise low-water soluble substrates (Roberts et al., 1995). However, enzyme isolation and purification can be quite expensive and time consuming, the addition of co-factors or their recycling may be required and, in general, it is more difficult to carry out reactions requiring more than one enzyme, contrarily to the use of whole microbial cells.

A large number of new enzymes and biosynthetic pathways have been reported by genome sequencing, increasing considerably the field of enzymology and permitting the development of drug design (Begley and Tsai, 2003). Application of computational methods to functional protein design allows optimization of engineering target activities but cannot identify subtle changes in protein structure necessary to improve activity (Bolon et al., 2002). On the other hand, directed evolution can improve activity and selectivity of existing enzymes but not create novel catalysts. Directed evolution of oxygenases to overcome their low stability, expression or activity has led to the development of P450s able to oxidize new substrates and function without biological co-factors, to an increased range of substrates of dioxygenases and to an increase of chloroperoxidase activity (Cirino and Arnold, 2002). The combination of both techniques, computational design and directed evolution, can improve dramatically the scope of *de novo* enzyme design (Bolon et al., 2002). Another approach to improve e.g. hydroxylation activity is substrate engineering, through which the substrate is modified to increase substrate acceptance, and regio- and stereoselectivity (de Raadt and Griengl, 2002).

Recently, cell free systems have been developed to carry out novel complex biochemical reactions *in vitro* (Bujara et al., 2010; Hold and Panke, 2009; Zhang, 2010). Using synthetic biology, cell free enzymatic biotransformation pathways are engineered by assembly of usually more than 10 purified enzymes and coenzymes for the production of the desired products that could not be produced by a single enzyme. However, and contrarily to whole cells, these systems are unable to self control and repair (Bujara et al., 2010).

The major advantages of using whole cells is that cells provide a natural environment for the enzymes, preventing conformational changes in the protein structure that would lead to loss of activity in non-conventional medium, and are able to efficiently regenerate co-factors (de Carvalho and da Fonseca, 2007). A single bacterial strain can also produce a wide array of intra- and/or extracellular enzymes, the production of each being dependent on the growth conditions and on the cellular development (Bode et al., 2002). Bode and co-workers isolated, from only six microorganisms, more than 100 compounds belonging to more than 25 different structural classes as a result of systematic alteration of cultivation parameters such as media composition and aeration (Bode et al., 2002). The Nature's diversity has arisen from microbial adaptation to Earth's different, and often extreme, micro-environments. Microorganisms learned to live in extremes of e.g. temperature, pH, pressure and salt. Growing these microorganisms in the laboratory might be difficult and diversity has been demonstrated by genetic assessment, in particular ribosomal analyses (Handelsman, 2004; Su et al., 2004). Metagenomics allow the study of the genome of environmental microorganisms that cannot be cultured under laboratorial conditions and through function-based or sequence-based screening of metagenomic DNA libraries, novel biocatalysts can be found (Lefevre et al., 2007; Schmidt et al., 1991; Streit et al., 2004). However, it was showed that only by complementing metagenomic techniques with parallel culture libraries can the full extent of microbial diversity within a community be understood (Donachie et al., 2007).

Processes that have been used, in some cases for centuries such as cheese and wine making, have been improved by the discovery and enhancement of biocatalysts and reaction conditions (Table 1). In this review, different methods of improving the stability and activity of both enzymes and whole cells in biocatalytic systems are discussed, as well as the use of extremophiles to find new metabolic routes and commercially interesting compounds. Several examples of biotransformations leading to the production and isolation of chiral compounds, probably one of the best applications of biocatalysis, are also presented.

Table 1
Improvement achieved by new methods applied to known processes.

Process	Method	Improvement	Reference
Cheese ripening	Genetically engineered starter cultures and/or recombinant enzymes	Faster cheese maturation	Azarnia et al. (2006) and Johnson and Lucey (2006)
Food products from starch (e.g. fructose, trehalose)	Recombinant enzyme technology and protein engineering	Starch hydrolysis and conversion to useful products	Akoh et al. (2008), Chen et al. (2006), and Fernando et al. (2006)
L-Ascorbic acid production	Metagenome-derived functional genes and enzymes	Higher catalytic efficiency and thermostability	Eschenfeldt et al. (2001)
Production of L-phenylalanine	Introduction of a stress-responsive gene in a recombinant *E. coli*	Higher yield and lower acetic acid production	Ojima et al. (2009)
Acetate	Engineered *E. coli*	Single-step process using glucose	Causey et al. (2003)
Ethanol production	Enzymes from (acido)thermophiles	Degradation of lignocellulosic biomass	Miller and Blum (2010)
Flavour compounds	Genetic engineering	Higher production yields	Schrader et al. (2004)
Pulp and paper industry	Enzymes from extremophiles (e.g. xylanases)	Reduce the chlorine necessary for pulp bleaching	Frock et al. (2010) and Jiang et al. (2006)
Human insulin and analogues	Production in recombinant strains and protein engineering	Higher production yields	Kafshnochi et al. (2010) and Walsh (2005)
Production of antibiotics	Genome mining	Discovery of novel products	Corre et al. (2008) and Zerikly and Challis (2009)
Fermentation and biocatalytic processes (e.g. antibiotic production)	Mechanistic modeling (e.g. metabolic flux analysis)	Development of efficient processes	Gernaey et al. (2010) and Sin et al. (2008)