Foreword

Frontiers in Biocatalysis

Biocatalysis, A Field of Exploding Interest

Biocatalysis can be defined as the application of a biocatalyst to achieve a desired conversion under controlled conditions in a bioreactor. A biocatalyst can be an enzyme, an enzyme complex, a cell organelle or a whole cell. Over recent years there has been an exponential increase in the production of high-value specialty chemicals using either isolated enzymes, especially hydrolases, to catalyze single-step transformations or whole cells to catalyze multi-step reactions. This increase is motivated by the fact that enzymes, whether isolated or contained within whole cells, can catalyze a broad range of reactions, at times with high levels of chemoselectivity, regioselectivity and stereoselectivity. In fact, biocatalysts in the form of isolated enzymes, or enzymes contained in whole cells, can often catalyze reactions with a specificity that is difficult to obtain by classical chemical routes. Further, they act under mild conditions of pH and temperature and biocatalytic processes generate fewer residues than chemical processes do, such that biocatalysis is often referred to as »green chemistry«.

The ability to carry out enantioselective transformations is possibly the most important factor that has motivated interest in biocatalytic processes. Over recent years there has been an immense number of publications about the enzymatic resolution of racemic mixtures or enzymatic synthesis of chiral compounds, stimulated by the demand of the pharmaceutical industry for enantiopure compounds. The total sales of chiral pharmaceuticals exceeded US$100 billion in 2000 and the market for enantiomerically pure or enantiomerically enriched organic compounds continues to expand rapidly *(1).* However, applications of stereoselective synthesis of chiral organic compounds are not limited to the pharmaceutical industry, they also extend to the production of agrochemicals, cosmetics and fine chemicals.

In recent years, many companies have established commercial biocatalytic processes for the production of chiral compounds, some examples being the production of chiral amines and alcohols at a 100 t scale by BASF using a lipase from *Burkholderia plantarii*, the generation of an intermediate for the synthesis of Diltiazem by DSM using a lipase from *Serratia marescens*, the production of *S*-Ibuprofen by Sepracor using a lipase from *Candida cylindracea* and the production of (*S*)-phenylalanine by Coca-Cola, using subtilisin Carlsberg *(2).* In this special issue, the production of chiral pharmaceutical compounds is reviewed in depth by Patel (*3*), while Alcántara *et al.* (*4*) address a crucial related topic, namely the reproducibility of results for enantioselectivity that are achieved when supposedly the same biocatalyst is used, but obtained from different suppliers or even from different lots of the same supplier.

Of course, pharmaceuticals are not the only products of biocatalysis. Two of the articles in this special issue address some quite different applications, namely the production of aroma compounds from lipids by Aguedo *et al*. (*5*) and the production of dextran and prebiotic oligosaccharides by Gómez de Segura *et al.* (*6*).

Novel Biocatalysts or Biocatalysts with Novel Properties?

Industrial applications are now demanding enzymes or microorganisms with »unnatural« characteristics, such as the ability to act on substrates other than their »natural« substrates and high stability and activity, not only in aqueous media but also in the so called »non-conventional media« or at high salt concentration or high temperature. However, since the aim of biocatalysis is often to produce a compound that is not produced by »normal« metabolic routes, finding a biocatalyst for a particular application is not necessarily an easy task.

There are essentially two approaches to choosing a biocatalyst: the screening for novel biocatalysts, taking advantage of the largely unexplored biodiversity that exists on our planet, and the screening for new activities amongst the existing biocatalysts. In the latter approach we can also include the improvement of known biocatalysts through protein engineering, either through molecular biology or direct modification of the protein, and the modification of biocatalyst properties by »media engineering«.

In both approaches to obtaining an appropriate biocatalyst, it is often necessary to screen a large number of samples for a desired activity, selectivity or stability. In this case it is essential to have an appropriate high-throughput screening method available. Within this special issue Reymond (*7*) gives several case studies that illustrate the challenges involved in developing such methods.

Searching for novel biocatalysts

The currently known microorganisms represent only a fraction of the species present in nature. For example, based on the rate of discovery of new species, it is estimated that we currently know only 0.2 to 0.6 % of bacterial species, 5 % of fungi species and at most 24 % of algal species *(8)*. Therefore mass screening programs have a reasonable chance of finding an enzyme with the properties required for a specific biocatalytic process. These screening programs can involve either the microorganisms themselves or DNA libraries, including those prepared from unculturable organisms and expressed in bacteria *(9)*.

Extremophilic microorganisms are attractive candidates for the production of novel natural biocatalysts. Many extremophiles belong to the archaea and contain various enzymes and cofactors not found in bacteria. However, although various enzymes have been isolated from hyperthermophilic, halophilic, piezophilic and psycrophilic organisms over the last fifteen years, as yet only relatively few have found commercial applications. Within this special issue, Gomes and Steiner (*10*) review the potential of obtaining new biocatalysts from extremophiles, including the difficulty of culturing these organisms at large scale and possible solutions to this challenge. Of course, one of the solutions is to clone and express the relevant gene in a fast-growing easily-culturable organism such as *Escherichia coli*. In fact, screening of DNA libraries obtained from extreme environments and expressed in *E. coli* offers a new approach for the identification of novel biocatalysts in extremophiles. After sequencing the genes in the library, it is possible to search for sequence alignment with corresponding genes of biocatalysts originating from known mesophiles.

Adapting or improving the existing biocatalysts

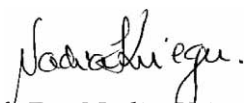
One can also search for new activities among known enzymes or microorganisms, including strains that are already used commercially. For instance, within this special issue Gonçalves *et al.* (*11*) show that microorganisms that are well known for the production of hydrolases, such as *Aspergillus niger* and *Aspergillus oryzae*, can also produce other enzymes under different conditions, such as epoxide-hydrolases. As another example, Rodrigues *et al*. (*12*) show that *Saccharomyces cerevisiae* can be used to catalyze various reduction reactions. Further, well known commercial enzymes, cheaply available and used in food processing, waste treatment and in the detergent, feed and textile industries, may exhibit activities that can be taken advantage of in biocatalysis. Such is the case of *Thermomyces lanuginosa* lipase, produced by Novozymes and used mainly in detergents. This enzyme can catalyze various different reactions under specific conditions *(13,14)*, such as the synthesis of ethyl-laurate in reverse micelles *(15)*.

Commercially available biocatalysts typically come from a relatively small number of bacterial and fungal species. Genera of *Aspergillus* and *Bacillus* tend to predominate the enzyme production industry, since they derive from ancient food processes and are Generally Recognized As Safe (GRAS) *(8)*. Improvements of the properties of their enzymes for biocatalytic purposes, such as improved temperature and pH stability and improved enantioselectivity, can be achieved either by modifying the enzyme molecule itself or the genes that encode for it (*protein engineering*) or by manipulation of the medium (*medium engineering)*. In this special issue Adamczak and Krishna (*16*) show the place of these strategies in research and development programs for the production of efficient biocatalysts. Krieger *et al*. (*17*) and Torres and Castro (*18*) complement their review, focusing on the use of heterogeneous and homogenous non-conventional media, respectively.

Higher eukaryotes also represent a potential source of biocatalysts, but the use of biocatalysts from these organisms has often been limited by the difficulties in production of large yields of enzymes from eukaryotes. The general potential for biocatalytic applications of enzymes from higher eukaryotes is explored in this special issue by Liu *et al.* (*19*), who point out that the cloning of these enzymes into microorganisms has opened up new possibilities. Fechter and Griengl (*20*) then review the potential of a specific group of enzymes produced by higher eukaryotes, namely hydroxynitrile lyases.

This special issue of *Food Technology and Biotechnology* on biocatalysis highlights the attention that biocatalysis is now attracting not only from academic research groups but also from industry. In fact, despite the great number of papers published on this topic, biocatalysis seems to be still in its infancy, with unlimited horizons ahead.

Guest editors:



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