

Biocatalysis Is a Microbial Field

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COLUMN ARTICLE

Humans have used enzymes for thousands of years in the form of fermentations to produce or preserve certain foods such as cheese, yogurt, vinegar, bread, wine or beer.

In the mid-nineteenth century, Louis Pasteur set an important milestone for enzymatic chemistry by developing a kinetic resolution by treating in aqueous solution a racemic mixture of tartaric acid with a culture of the fungus *Penicillium glaucum*, which consumed only the (+) enantiomer and thus an enrichment of the (-) enantiomer was obtained. Few years later, at the end of the nineteenth century, Eduard Buchner reported the fermentation of sugar using acellular extracts of yeast, demonstrating that to obtain biotransformations the living cells are not necessary.

Already during the first half of the 20th century, a new biocatalysis, in which fermentations with whole cells, extracts, or partially purified enzymes were used to explore a wide range of products both chiral like non-chiral, was developed.

However, since the beginning of its use, the enzymes used as biocatalysts in organic synthesis have suffered from two main drawbacks, their availability in large quantities that allow their industrial use and their narrow (in certain cases) range of recognition of substrates.

These two drawbacks have been gradually overcome in recent decades. The development of recombinant DNA technology since the late 1970s has enabled the production of both laboratory and industrial scale useful enzymes for synthetic chemistry, mainly applied in pharmaceutical and industrial chemistry. Secondly, the development of new techniques of molecular biology and molecular modeling has allowed the adaptation and improvement of natural enzymes to solve specific problems in synthetic routes of products of interest since the 1990s. These techniques have allowed the engineering of enzymes by directed evolution, saturation mutagenesis, gene shuffling or random mutation and high performance selection, among others, such as the current CRISPR/Cas9 techniques or the discipline of synthetic biology.

To date, thousands of enzymes have been described with different applicability in industry. Although the fields of theoretical application of biocatalysis are practically all of the chemical disciplines, it has undoubtedly been the pharmaceutical field where they have aroused greater interest, both in terms of their technical possibilities and their compatibility of scale of production and capacity to assume the cost of the process. In spite of this, there are a whole series of requirements that must be satisfied beyond the actual operability of the enzyme. That is to say, it is not enough for a given enzyme to be able to carry out a concrete reaction so that this concludes with a real process, scalable, economically profitable and, finally, able to transfer all the benefits of biocatalysis to society.

Some of these requirements have to do with the economic viability of the process, a fundamental parameter for private companies, such as product concentrations and the cost of the enzyme in the process. As for the concentrations of product obtained, they are normally expected to move between the usual values in traditional chemical processes of at least 50-100 g/L. Taking into account that enzymes work in cells at millimolar concentrations, this is not always easy to achieve the stated objectives. However, protein engineering and process development can help achieve such product levels without inhibiting enzyme activity in certain cases. As for the cost of the enzyme, it is usually expressed as “gram of product / gram of the biocatalyst” and this metric should reach at least a value of 1000 for purified enzymes and 15 for an entire cell system. Although, of course, these values are generalizations and should be considered in each particular case since the price of raw materials or products can end up determining the viability or not of a certain process in certain cases even above the price that can reach the enzyme itself. In any case, there are certain solutions that can be considered in general, such as the immobilization of the enzymes in some way that easily allows their recovery at the end of the process and its reuse, in addition to allowing a better recovery of products.

Beyond the advantages that a biocatalytic process can provide for an industrial production compared to a traditional process, it should be pointed out that, even within the world of biocatalysts, great differences can be found between enzymes, not only because of their functionality, but also because of their capacity to be supplied on an industrial scale. In this sense, the development of industrial enzymes has depended heavily on the use of microbial enzymes. Microbes are useful because they can be produced economically in short fermentations on cheap media, their tracking is simple and the improvement of the strains for better yields has been very successful. In the 1980s and 1990s many microbial enzymes replaced animal or plant enzymes in many industrial applications, including food (human and animal), detergents, textiles, fur treatment, paper industry, or enzymes for diagnosis and therapy. In addition to natural microbial enzymes, the development of recombinant DNA technology had a huge impact on the levels of enzyme production on a global scale, since a large number of enzymes

have been produced, regardless of the species of origin, in industrial microbial strains.

This technology has been rapidly accepted by industry because it can increase hundreds of times the productivity of an enzyme with respect to its natural levels and because it allows the production of enzymes from strains not suitable for industry in industrial strains. More than 50% of the enzymes present on the market are recombinant enzymes. In many cases these enzymes have been used to substitute chemical synthesis routes. The increased use of enzymes in industry has involved either the use of purified or semi-purified isolated enzymes as well as the use of whole cells to carry out bioconversions.

Many enzymes can be operated at room temperature, under neutral aqueous conditions and in the absence of functional group protection. In organic synthesis, these biocatalysts can be used as the sole reaction catalyst, in combination with other enzymes, or with non-biological reagents. The chiral nature of the enzymes favors the synthesis of compounds with defined regio- and stereospecificities. In many cases the enzymes are able to recognize non-natural substrates and, in addition, genetic/enzymatic engineering may allow to improve their properties such as stability, substrate specificity, or increase their activity. The use of enzymes can, not only improve certain reactions, but allow specific modifications in molecules that, because of their complexity, cannot be modified by traditional chemical methods. Thus, the use of enzymes in industrial or pharmaceutical chemistry represents an opportunity for the development of new processes.

For all these reasons, despite the great challenges ahead, the health of biocatalysis today is better than ever. It is a mature discipline but still in full expansion and, although certain applications have been described in non-microbial models, biocatalysis remains mostly microbial. The ease of obtaining, handling and production, the lack of moral connotations and the new technologies that continue facilitating the engineering of the strains and the purification of the products make it possible to predict that the production of biocatalysts for use in industry will remain a fertile field for microbiology [1-10].

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