Back to the future: taking enzymes to the next level of sustainability

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The use of enzymes (protein catalysts from biological origin) has been key to the development of our society and daily life since the dawn of humanity. Nowadays, the better understanding of how enzymes work and their manipulation has enabled enzymes to become a crucial technology in the current biotechnological revolution. In this sense, while enzymes in their naturally occurring form are excellent biocatalysts, they are not yet broadly implemented in industry due to their instability and poor reusability. As a solution, enzyme immobilization is a strategy that enables the preparation of more resistant, reusable and more cost-efficient biocatalysts that, combined with continuous flow technologies, have the potential to make their promise true: transition towards more cost-efficient, sustainable, and environmental friendly chemical manufacturing.

Millennia ago in the Middle East, specifically in Egypt, our Neolithic ancestors started to make bread and beer. This was the first time that humans applied enzymes to manufacture valuable products, even though they were not aware of their existence yet. The addition of yeast, a living microorganism which contains enzymes, is needed for the fermentation to make the dough, in the case of bread, or to produce alcohol in beer.

Enzymes are natural protein catalysts that transform substrates, such as sugars in the fermentations, into products. This process is the key concept of biocatalysis: the use of enzymes to speed up biochemical reactions by facilitating molecular rearrangements (Figure 1). In every cell of our body and in all other organisms, there are more than 1300 different enzymes working as coordinated nanomachines with specific functions to sustain life.

The green industrial revolution

Nowadays, enzymatically produced commodities are ubiquitous in our daily life, from the juice we drink for breakfast to the detergents we add to do the laundry (Figure 1). If you have never done it, go and read the label of your laundry detergent bottle, you may find 'it contains enzymes'. Nevertheless, the application of enzymes is not always so straightforward. Enzymes are adapted to their natural environment (physiological pH, temperature, salinity), which might be rather different from the conditions employed in industry (presence of organic solvents, extreme temperatures). Then, why bother using enzymes in industry? Indeed, many catalytic processes have been typically carried out by using chemical catalysts that are synthetically produced. However, those chemical catalysts present disadvantages that are augmented at the large scale applied in industry. Compared to chemical catalysts, enzymes offer a higher selectivity for the substrate, avoiding undesired by-products and cross-reactivity between different substrates. Moreover, enzymes allow for a reduction in the quantity of catalyst needed to catalyse a reaction and therefore the process costs. In addition, enzymes are biocompatible and biodegradable catalysts, facilitating the waste treatment after their disposal and decreasing the use of toxic reagents, which is especially important for the manufacturing of products for human consumption. Overall, enzymes as biocatalysts are an eco-friendlier alternative to the traditional chemical synthesis, reducing waste and investment on resources.

Although enzymes generally work under mild (physiological-like) conditions, we can engineer them, meaning that we can modify their protein structure, so that they can work in harsher environments. Likewise, enzymes can be engineered so as to broaden the scope of substrate molecules that can accept, increasing the range of biochemical reactions that they catalyse. Enzyme engineering has truly revolutionized the enzyme world during the last decades, enabling the design and production of enzymes on demand. To achieve that, bioinformatic analyses are a key guide to predict the behaviour of engineered enzymes and to rapidly analyse numerous enzymes simultaneously. Hence, we can provide a more sustainable approach to reduce

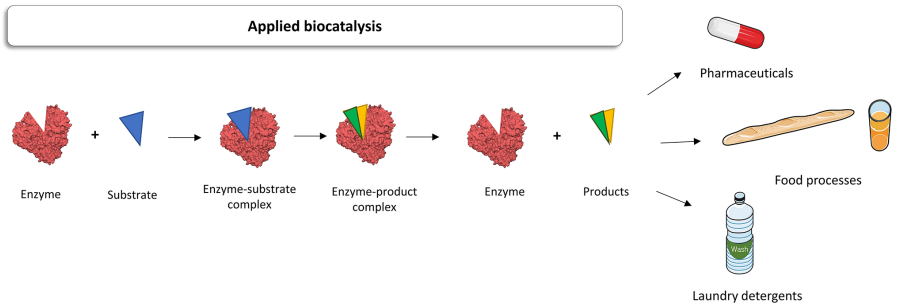

Figure 1. Enzyme catalysis process to transform substrates into valuable products

costs, time and waste that would otherwise be generated by performing those time-consuming analyses in the laboratory. The future is bright for enzyme engineering, and with increasing knowledge and capacities we will untap the potential of enzymes to manufacture more complex molecules such as pharmaceuticals in the next years.

How do we measure sustainability?

It was not until the 1980s, when our society started to be concerned about the generation of waste and the use of hazardous reagents in chemical manufacturing. This was the trigger for hunting novel and cleaner alternatives, such as the application of enzymes in catalysis. Therefore, the concept of sustainable chemistry (aka green chemistry) emerged and, from its foundations, it was defined as:

- The efficient use of raw (and preferably renewable) materials and energy resources for the manufacturing and application of chemicals.
- Eradication of waste and use of hazardous reagents resources for the manufacturing and application of chemicals.

With the clear concept, all we need are metrics that help us to assess how sustainable is an enzymatic process compared to other routes. To this end, the two most well-known and easiest green metrics are:

- *E* factor: relation between the waste generated during the process and the amount of final product obtained. The lower the *E* factor is, the more sustainable is the process. Ideally, the *E* factor should be as close as possible to zero:

$$E\,factor = \frac{\text{kg of waste}}{\text{kg of product}}$$

- Atom economy (AE): theoretical value used as a predictive tool to quickly evaluate the waste that will be produced. The closer to 100%, the most sustainable is the process as it translates into no loss of starting materials.

$$AE\ (\%) = \frac{M_w \text{ of the desired product}}{\text{sum of the } M_w \text{ of starting materials}}$$

M_W refers to the molecular weight.

Immobilized to move forward

One of the strategies that have emerged over the last decades to make enzymes more robust and greener biocatalysts is enzyme immobilization. The concept is very simple: enzymes molecules are trapped inside a solid material (Figure 2). You can imagine this strategy as providing a huge turtle shell to protect many small turtles. As an alternative, enzyme immobilization can also be performed by attaching enzymes to a solid material or cross-linking the individual enzymes. Immobilized enzymes are more stable and resistant to extreme conditions like those common in industrial setups. Additionally, immobilized enzyme can be easily separated from the products by simple filtration

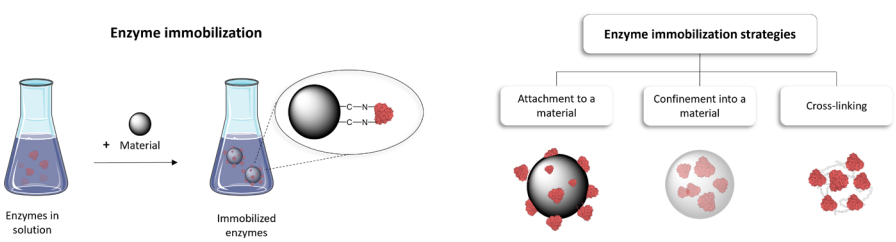

Figure 2. Enzyme immobilization. On the left, the procedure to immobilize enzymes is depicted. The example shows an irreversible immobilization. On the right, the three main strategies to immobilize enzymes.

facilitating the product purification and enabling the reuse/recycle of the enzyme for a new reaction. Furthermore, as immobilized enzymes become a bigger particle, they can be integrated into flow reactors.

Yet, there is still a long road ahead of enzyme immobilization towards sustainability, and the holy grail: a universal immobilization technique. The most used materials to attach enzymes are microbeads made of methacrylate, agarose, silica, glass, or other polymers (i.e., polystyrene). Most of them are very costly and cannot be easily degraded, thus producing more waste. Furthermore, enzymes are typically bound to the solid material in an irreversible manner to prevent the leaching of enzymes to the media during the reaction that might end contaminating the final product. Therefore, once the enzyme loses its activity over time, both the enzyme and the material go to the waste bin. To tackle those issues, new research directions towards green materials and reversible immobilization binding strategies are emerging. For instance, lignin derivatives are an attractive substitute to the fossil-based materials for enzyme immobilization as lignin biomass is obtained from renewable sources, is biodegradable, and non-toxic.

Let's go with the flow

As mentioned before, one of the options immobilized enzymes unlock for us is their use in continuous systems. Continuous flow chemistry, which originated as a branch of organometallic chemistry, enabled an easier handling of hazardous reagents and minimization of the reaction. But soon enough, this continuous production crossed over to other fields. While in the typical batch setup the reagents are mixed first, stirred for a certain time and then discharged from the vessel to obtain a certain amount of product, in continuous flow the mixing and reaction happen in the 'pipes' and the product is obtained non-stop (as long as initial solutions are provided, of course). With enzymes, the problem to use them in flow is that they are homogeneous catalysts (i.e., they are in solution and cannot be recovered) but, alas, with enzyme immobilization we have solved this problem already as immobilized enzymes can be packed into columns (packed bed reactors or PBRs) that look like cartridges (Figure 3). The PBRs can be connected to the continuous flow machine (the simplest, a pump) to continuously provide starting material for the enzyme to transform. This is flow biocatalysis.

The beauty of this approach is its tuneability and flexibility: you can combine multiple cartridges to perform several transformations sequentially. You can change the cartridge to create completely different products from the same starting material and, more importantly, you can have an automated control of the reaction tailored to each cartridge in terms of temperature or pH, ensuring each enzyme works at its best. Downstream processing can also be coupled to the flow reaction, therefore obtaining the pure product in excellent quantities ($kg_{product}$/day) and superior catalyst productivity (as the immobilized enzyme is reused multiple times).

Flow biocatalysis promises a brighter, greener and more cost-efficient future to produce chemicals in our daily life, and industry is already looking with enthusiasm at the latest developments in the field. For example, pharmaceutical companies could benefit from the advantages of flow biocatalysis while complying with the FDA (Food and Drug Administration) and EMA (European Medicines Agency) requirements. Many active pharmaceutical ingredients and natural products

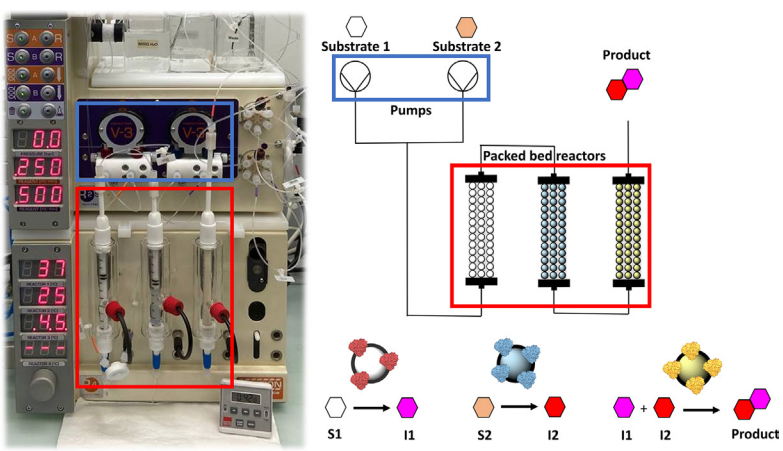

Figure 3. Flow biocatalysis. On the left, the actual flow chemistry machine is shown. On the right, a three-step enzymatic cascade is depicted in the schematic form. S: substrate, I: intermediate. In the example, the first packed bed reactor converts substrate one and, after conversion of the second substrate by the second PBR, the third catalyses their linkage to yield the final product.

have already been synthesized through flow biocatalysis at lab scale, and its implementation in industry will certainly reduce production costs. Therefore, consumers will experience broader access to pharmaceuticals at lower costs, making the drug market more accessible for low-income regions. ■

Further reading

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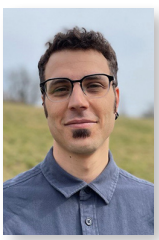

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