Biocatalysis for future sustainable manufacturing

John M. Woodley
(Department of Chemical and Biochemical Engineering, Technical University of Denmark, Denmark)

Enzymes are the catalytically active proteins, responsible for carrying out biochemistry in nature. Today, they are also finding use as catalysts in organic chemistry, both in the laboratory as well as in large-scale manufacturing of chemicals in industry. Their special properties enable sustainable syntheses, supported by tools such as protein engineering so they can be tuned to operate efficiently, thereby meeting industrial requirements.

Introduction

In recent years, the increasing population and climate change, together with geo-political instability, have all made clear how very important it is that we move to a more sustainable use of finite resources. In particular, processes for the large-scale production of food, chemicals and pharmaceuticals all require new thinking based on sustainable raw materials and using environmentally friendly processes. Raw materials in the near future will need to be renewable, relatively cheap and readily available (i.e., not in competition with existing markets). For example, renewable materials such as glucose are useful as potential raw materials, but are not sustainable unless extracted from waste materials, such as ligno-cellulosic waste from agriculture. Waste gases, such as CO_2, would be even more useful as potential raw materials but are often present at low concentrations.

These challenges for future raw material sources also drive another important topic in sustainability: the effective transformation of such materials into valuable products, using reaction technology. Today, many reaction technologies have been developed based on conventional catalysis, which is not always as selective as we would wish and is often best used at high temperatures and pressures. Frequently, therefore, such processes are not optimal from the perspective of environmentally friendly production, using a significant amount of energy and producing a significant amount of waste. For all these reasons, many researchers in academia and industry are now focused on biological reactions, which come with a host of advantages. For example, fermentation processes are good at converting renewable feedstocks, such as glucose, into valuable products at ambient temperature and pressure. However, obtaining higher selectivity is difficult using fermentation since the cell contains many enzymes, so by-products are almost inevitable. Alternatively, those enzymes which are specifically required for the reactions of interest can be extracted from microbial cells and used as isolated enzymes. The use of these isolated enzymes for organic synthesis or industrial chemistry is termed biocatalysis.

Biocatalysis

Enzymes, which are catalytically active proteins, are often described as nature's catalysts and help speed up reactions under mild conditions in all aspects of life on Earth, from mammalian cells to microorganisms. This extraordinary ability to catalyse the most important chemical reactions for life is combined with exquisite selectivity due to the very large size of these dynamic proteins (typically ranging from 20 to 200 kDa). For the last 50 years or so, this ability has become of greater and greater interest to organic chemists, tasked with producing complex molecular structures (either for fundamental research or for application in industrial production processes). In particular, in the pharmaceutical industry, developments of such syntheses are accompanied by significant development costs and result in complex processes. For this reason, biochemists together with organic chemists started to realize that beyond the fascinating study of enzymology, enzymes could also be very useful as reaction catalysts. This has driven the new discipline of biocatalysis, where enzymes are used as a catalytic tool. Today, this growing field has attracted many thousands of researchers and has recently given rise to a Nobel Prize winner : Frances Arnold (USA). In 2018, she was awarded one half of the Nobel Prize for Chemistry for discovering that by changing the amino acids which make up the enzyme, its properties could be changed, and even tuned to our liking. It is hard to overstate the power of this discovery and its associated methodological inventions, inspired by the evolutionary approach used by nature itself. In this way, enormous progress has been made in altering the functional properties of various enzymes to match the fundamental needs of the organic chemist. In fact, entirely new reactions have even been identified (see further reading, Chen and Arnold (2020)). This very special ability provides great potential for the application of enzymes in synthesis and production.

Moving into manufacturing

Translating reactions in the laboratory into industrial processes is not simple and demands a focus on the economy of the process to ensure that the cost of raw materials, catalysts and utilities allows enough economic margin for paying back capital and making a profit. The lower the price of the product, the harder this is to achieve. Biocatalysis also needs to fulfil these basic production rules, but comes with the special ability to protein-engineer the enzyme to have the desired properties for a given process. In principle, the ability to protein-engineer an enzyme means that high concentrations of substrate can be used and high concentrations of product can be produced, at an adequate rate and under optimal conditions. However, this also demands extra time for biocatalyst development.

Enzymes are catalysts, meaning they are unchanged during the reaction and therefore can be added in extra amounts to help reactions go faster. Double the enzyme concentration gives double the rate of reaction and so on. This is particularly important in the large-scale manufacturing of low-priced products (less than 20 USD/kg), because of the quantity of product required to meet market demands (greater than 20,000 t/year). As in other cases, since the size of the process is directly linked to the rate of the reaction, the faster the rate the smaller the plant. If the enzyme has sufficient activity, then it can easily be possible to achieve adequate rates (greater than 20 g/L/h). However, it is also important to ensure that not too much enzyme is added, since this is expensive. One way to minimize the amount of enzyme added is to recycle the biocatalyst and use it many times. This necessitates an adequate stability (structural integrity as well as maintaining the initial rate of reaction over time). Further work is still required on stability.

New-to-nature enzymology

Beyond the extraordinary ability to scale- up and use enzymes for manufacture of valuable products, assisted by protein engineering lie some deeper questions. To date, we have only explored a small fraction of life on our planet and alongside this only a small fraction of possible enzyme structures. In fact, altering enzymes via directed evolution could result in too many configurations to test. For this reason, computer-aided approaches, including machine learning, increasingly play a role in defining suitable libraries of catalytically active proteins for testing. Still it is necessary to define the conditions suitable for testing. Here we need to operate in conditions far from those normally found in nature, with new substrates, new concentrations and, e.g., unusual media with air bubbles, containing oxygen for oxidation reactions. It may yet prove that some of these conditions can be found in remote locations on Earth, but for now the task is to find enzymes stable under these very unusual conditions found inside industrial reactors. Figure 1 schematically illustrates some of the tasks which need to be addressed to develop new-to-nature enzymology.

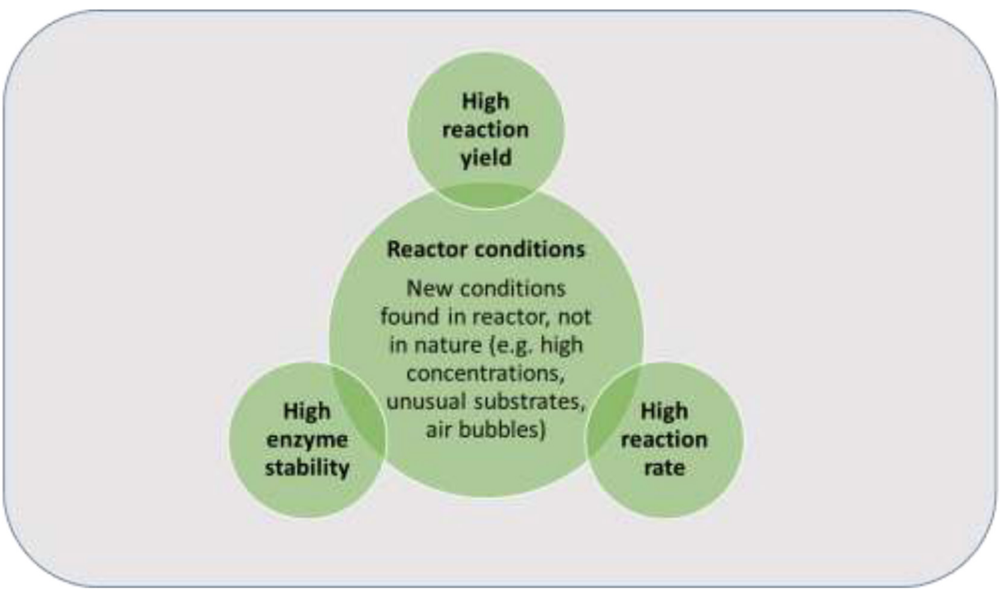

Figure 1. New-to-nature enzymology.

Cascading enzymes

In biocatalysis, an individual enzyme converts a reactant (most commonly termed a substrate) into a product, and each enzyme carries out a specific type of reaction, inserting, removing or shuttling a single functional group between one or more substrate and product molecules. Hence, by combining many enzymes in a pre-defined sequence, a novel pathway can be created. The majority of enzymes operate under relatively similar mild conditions of pH, temperature and pressure so they can even be combined in a single reactor. Such novel pathways are usually in the form of a cascade where the product of one reaction acts as the substrate for the subsequent one and so on. Such cascades also exist inside living cells, but are constrained by local conditions. Today, *de novo* cascades of isolated enzymes have been constructed of several enzymes together suitable for industrial scale-up. Among the advantages of cascades is the ability to pull reactions to completion using Le Chatelier's principle where the reaction equilibrium might otherwise be unfavourable. Here the second reaction pulls the product from the first reaction. Such a principle can also be used where the product has an inhibitory effect on the enzyme, lowering the rate of reaction at higher concentration. Here too the second enzyme can pull the product from the first reaction, provided the second reaction is fast enough. Cascades, like these, are already used to make pharmaceuticals, avoiding otherwise long syntheses. (An example is given in the suggested further reading, Huffman *et al* (2019).) The hope now is to use such cascades for lower-priced bulk chemical products where the benefits for sustainability are even greater.

A bright future

The realization of many new and sustainable manufacturing processes will be possible using new enzyme activities, tuneable by protein engineering. Interestingly, artificial enzymes and catalytic antibodies have also been shown to catalyse new reactions although currently at relatively low catalytic efficiencies. A key requirement for further success in enzyme-based catalysis in the future will be the need for still closer interdisciplinary work, where bio-scientists work very closely with process scientists and engineers to develop new biocatalysts and new processes hand-in-hand. An essential part of this will be to find a common language so all talk about the same requirements for biocatalyst and process. That is not only very valuable in our quest for a sustainable future, but also very exciting. ■

Further reading

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John Woodley obtained his undergraduate degree from UMIST (Manchester, UK) in Chemical Engineering and PhD in Biochemical Engineering from UCL (London, UK) in 1988. He is currently Professor of Bioprocess Engineering at DTU Chemical Engineering in Denmark, where he has been since 2007. His research interests are in the development of novel bioprocesses, with particular focus on biocatalysis, encompassing work on kinetics, thermodynamics and enzyme stability. Current enzymes of interest include oxidases, oxygenases, transaminases and lipases. Email: jw@kt.dtu.dk

