ACCELERATING CHEMICAL DEVELOPMENT AND MINIMIZING COST FOR FINE CHEMICAL/API MANUFACTURE AND METABOLITE SYNTHESIS

The wealth of information regarding enzyme technology and development is growing exponentially, and biocatalysis technology has now become one of the first choice methods in the fine chemical and pharmaceutical industry. Given the severe pressure to lower costs, minimize waste and shorten existing syntheses, the industry is in need of economic, robust, scaleable and reliable processes for manufacturing chiral APIs and intermediates. This need has resulted in process chemists utilizing their skills at the interface of chemistry and biology, and embracing biocatalysts and biocatalytic processes in organic synthesis. This paradigm shift has resulted in biocatalysis becoming the work-horse of the chemists’ tool-box for chiral chemistry.

Why has there been this surge in the application of this technology? The answer is simple: success breeds success. The key difference between biocatalysis today compared with 10 years ago is the availability of all the supporting technologies, such as bioinformatics, enzyme evolution and high-throughput screening, which make a real difference in enzyme development. Processes can now commence within weeks, and enzymes evolved in months.

The rapid implementation, economic benefits and ‘green’ reputation of biocatalysis provide superior solutions for solving complex chemistry problems. Enzymes have numerous advantages in this field, particularly their ability to discriminate between subtle differences in shape and functionality, either within a given molecule or in a mixture of compounds. This allows selective chemistry, for example, regio-selectively transforming a single functionality (out of several) in a single molecule or, more importantly, effecting the biotransformation of one isomer in a racemic mixture, facilitating its separation into the component isomers. Furthermore, enzymes have been used successfully in the scale-up of asymmetric processes, particularly in chiral alcohol and amine production, and for accessing difficult-to-synthesize metabolites.

Biocatalysts are unsurpassable when it comes to selectivity and distinguishing between subtle differences within a molecule. It has been shown repeatedly that speed of enzyme identification and scale-up is critical in demonstrating to customers that biocatalysis will compete with other technologies in respect to the cost of goods and development. At Almac, for example, the selectAZyme platform for enzyme identification is used. Typical timelines required from selection of a selectAZyme catalyst to actual manufacture of product is similar to that of conventional chemistry optimization and scale-up (Figure 1).
Hydrolases and carbonyl reductases continue to dominate the press and application stream. Enzymes such as transaminase and oxidative enzymes are growing in influence, because they become more tolerant to extreme conditions; that is, have higher substrate affinities translating to high activities, compatibility with organic co-solvents, tolerance of increased temperatures, and the availability of practically applicable and economical cofactor recycle systems.

In many cases, the development and scale-up of bioreolution and bioreduction have become routine, accessing hundreds of kilos of product in timelines comparable, if not shorter, to that of alternative chemical routes. The next milestone is in bio-oxidation. Increasingly, it is being applied to metabolite syntheses and has been proven to be the superior solution in accessing those metabolite that are difficult-to-synthesize. The marriage of microbial and recombinant enzymes is a real powerhouse in accessing difficult-to-synthesize metabolites. The scale-up capability allows the company to rapidly take ‘hits’ from conception to gram delivery as and when the customer requires the products. In addition, radiolabelled versions of these metabolites can also be supplied, utilizing the in-house isotope chemistry facility.

A typical project for a bio-oxidation is shown in Figure 2 and was initiated with selectAZyme platform screening. Following a successful screening project, which identified an active P450 enzyme (AL-103) for the transformation, Almac was contracted to generate 15 g of the API metabolite. Expression of the desired P450 in E. coli enabled the growth of the required biomass in a 150-L fermentation vessel after brief optimization at shake flask scale. A finding of particular note was the large beneficial effect of supplementing the growth medium with Fe(III)Cl₃ during the expression phase. Process development studies indicated that the dissolved oxygen level and the substrate addition rate were key parameters to control the accumulation of acceptable product concentrations. Specifically, it was found that dissolved oxygen levels needed to be maintained above 70%, whilst an addition rate of 25 mL/h of a 0.5 g/L solution of substrate in dimethyl sulfoxide could not be exceeded. Higher addition rates resulted in the formation of an insoluble polymorph that was completely incompatible with the biocatalyst. Overall yield for this process following purification by filtration through a silica pad was 61%.

There has been a paradigm shift in the acceptance of biocatalysis as a powerful tool at the forefront of process research within the industry. This is being driven by market needs and stresses, and key for future success are flexibility and rapid response to change. Biocatalysis is a maturing technology and will play an increasingly pivotal role in future success with the rapid supply and delivery of chiral intermediates, fine chemicals and APIs.