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Pharmaceuticals

Genes to GMP: Biocatalysis in the pharmaceutical industry today

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Stefan Mix of Almac Group looks at how a once niche technology has gone mainstream*

Biocatalysis is no longer considered an emerging technology in the pharmaceutical industry; it has taken a firm foothold in the form of numerous manufacturing processes, with applications ranging from pre-clinical development batches to commercial API manufacture. The rise of biocatalysis is inextricably linked to the ease of access to the enzymes it relies on.

The roots of pharmaceutical enzymatic chemistry can be traced back to the 1950s, when whole-cell microbes were used for steroid oxidation.1 Since the 1970s, the industrialisation of detergent enzymes has led to a strong uptake of lipases, esterases and proteases as catalysts by organic chemists. The attraction to enzymes is largely because of their unrivalled selectivity and specificity of reaction product formation, and because there is no need to use costly protecting groups.

Early days

In its early days, biocatalysis was an exotic niche technology. Its true emergence as a versatile technology class came with recombinant expression and directed evolution in the 1990s. This coincided with the genetic mapping of the biosphere, enabling advances in information technology, but also new competition in the form of asymmetric transition metal catalysis.

The cost of new enzymes was still comparable to the cost of a new metal ligand system, confining biocatalysis to large-scale applications with sufficient investment backing. Examples included bio-hydroxylation and bio-hydrocyanation processes developed and run by the likes of DSM and Degussa, who invested in either catalysis technology as appropriate.[2,3]

This changed when a whole new supplier landscape established to meet the needs of biotechnology – the falling cost of genes, increased database access, rapid and inexpensive sourcing of essential consumables, such as primers and restriction enzymes - enabling biocatalysis to flourish in among more than just a few big players.

From niche to first choice option

As most large pharma companies invested in dedicated biocatalysis laboratories their output multiplied by using provisions from specialised developers of enzymes instead of having to clone all enzymes themselves.

For example, carbonyl reductase (CRED, also known as alcohol dehydrogenase) technology began to outcompete rival metal catalysis systems. Their attraction was in the value of new stereocentres needed in new drugs. The abundance and robust versatility of natural reductase enzymes meant finding a biocatalyst for an asymmetric reduction became much easier, quicker and cheaper than developing a metal ligand system. The list of examples of their utility has been growing ever since.[4]

Other enzyme classes were also developed and, where natural diversity does not offer industrialisation-ready enzymes, protein engineering can often add the missing performance features.[5]

Abundance & diversity

It is not easy to find accurate statistics on numbers of biocatalysis applications in the pharmaceutical industry. The approach taken here was to use the number of biocatalysis publications in Organic Process Research & Development (OPR&D), an industrial organic chemistry journal mainly focused on pharmaceutical manufacturing and process development.

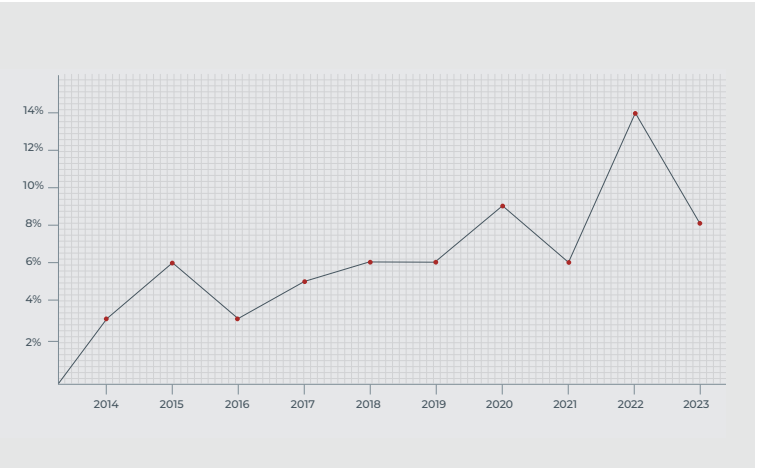
Figure 1 shows a snapshot of industrial biocatalysis R&D activity and tracker of application development. This demonstrates a sustained growth of numbers and total publication share. What the statistic does not show is the diversity of the publications, but it demonstrates that biocatalysis is no longer a niche or emergent technology.

Nowadays, most pharmaceutical CDMOs have dedicated biocatalysis groups. Those like Almac, who have been leading this trend for over 15 years, have amassed huge libraries of enzymes from many classes to facilitate the need for speed in early clinical development.

Almac has also invested in metagenomics, a technique to access more of nature's enzyme diversity, helping to advance enzyme engineering from a place not well suited to quick problem solving to a better place, where the power of computation and rational prediction replaces the need for slow and costly handling of tens of thousands of colonies and clones.[6]

Fig 1 : **Percentage of ORPD biocatalysis related publications**

Year	Percentage of OPRD publications
2014	3%
2015	6%
2016	3%
2017	5%
2018	6%
2019	6%
2020	9%
2021	6%
2022	14%
2023	8%



Regularity & novelty

While many enzyme classes have been investigated and many more will follow, Almac statistics indicate their application frequency and magnitude. The top spot is still held by CRED enzymes, with the wider reductase category also featuring in manufacturing with amino acid dehydrogenases (AADHs) and imine reductases.

Interestingly, the fragility of oxidative enzymes has been overcome for Bayer-Villiger-monooxygenases (BVMO), and scale-up applications now range from small- and large-scale preparation of non-GMP building blocks and regulatory starting materials to late-phase and commercial API manufacture with biocatalytic route-defining steps.

Reductases are the most frequently used enzyme class because of their reliability and predictability, making their selection a low-risk option. AADH enzymes demonstrate the versatility and substrate promiscuity of this class by enabling access to unnatural amino acids, which have become ever more frequent building blocks for new drugs, and feature as key ingredients in the trend towards larger drug molecules, such as peptides.

Ligase technology

Recently, RNA-based therapeutics and vaccines have emerged as a powerful new treatment and prevention options for severe disease and illness limiting the human life span. RNA ligases are a powerful enzyme class, enabling the industrialisation and scale-up of these highly diverse large molecular entities.[7]

Whilst established solid-phase supported chemical synthesis methods can access a huge structural variety and molecular size of oligonucleotides, the cost of making long RNA this way is prohibitive for mass market application, due to the problematic nature of highly similar impurities and the need for preparative chromatography for clean-up.

RNA ligases can recognise their substrates among similar compounds and provide ligation and purification in one reaction step. Their rapid engineering with today's smart mutant library toolbox makes catalyst tuning towards the acceptance of unnatural, such as methylated or fluorinated, nucleosides a routine undertaking.

Fig 2 : **Recent multi-mg to tonne scale history**

Enzyme class	Product / Quantity / Number completed
Carbonyl reductase	RSM = 22 / GMP steps = 9 / Enzyme supply = 12
Lipase / Esterase	RSM =8 / GMP steps = 2 / Enzyme supply = 3
Amino acid dehydrogenase	RSM = 4 / GMP steps = 2 / Enzyme supply = 2
Imine reductase	RSM = 3 / GMP steps = 3 / Enzyme supply = 1
Transaminase	RSM = 2 / GMP steps = 1 / Enzyme supply = 1
BDMO	RSM = 1 / GMP steps = 2
Liopxygenase	GMP steps = 1
P450 / other hydroxylate	RSM = 2
Late Phase / Commercial	GMP steps = 4 / Enzyme supply = 5

Outlook

From a biocatalysis expert CDMO perspective, the trends of today that will become the treatment agents of tomorrow include:

- Further roll-out of robust chiral technologies, including more and more oxidation, for instance producing chiral sulfoxide pharmacophores
- Overcoming equilibrium problems in bio-amination technology by developing high affinity co-enzyme systems
- Further development of peptide ligation to enable mass production of peptide APIs with unnatural fragments that cannot be produced by recombinant fermentation. Innovations in co-factor regeneration are also emerging to help to reduce cost.[8,9]

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