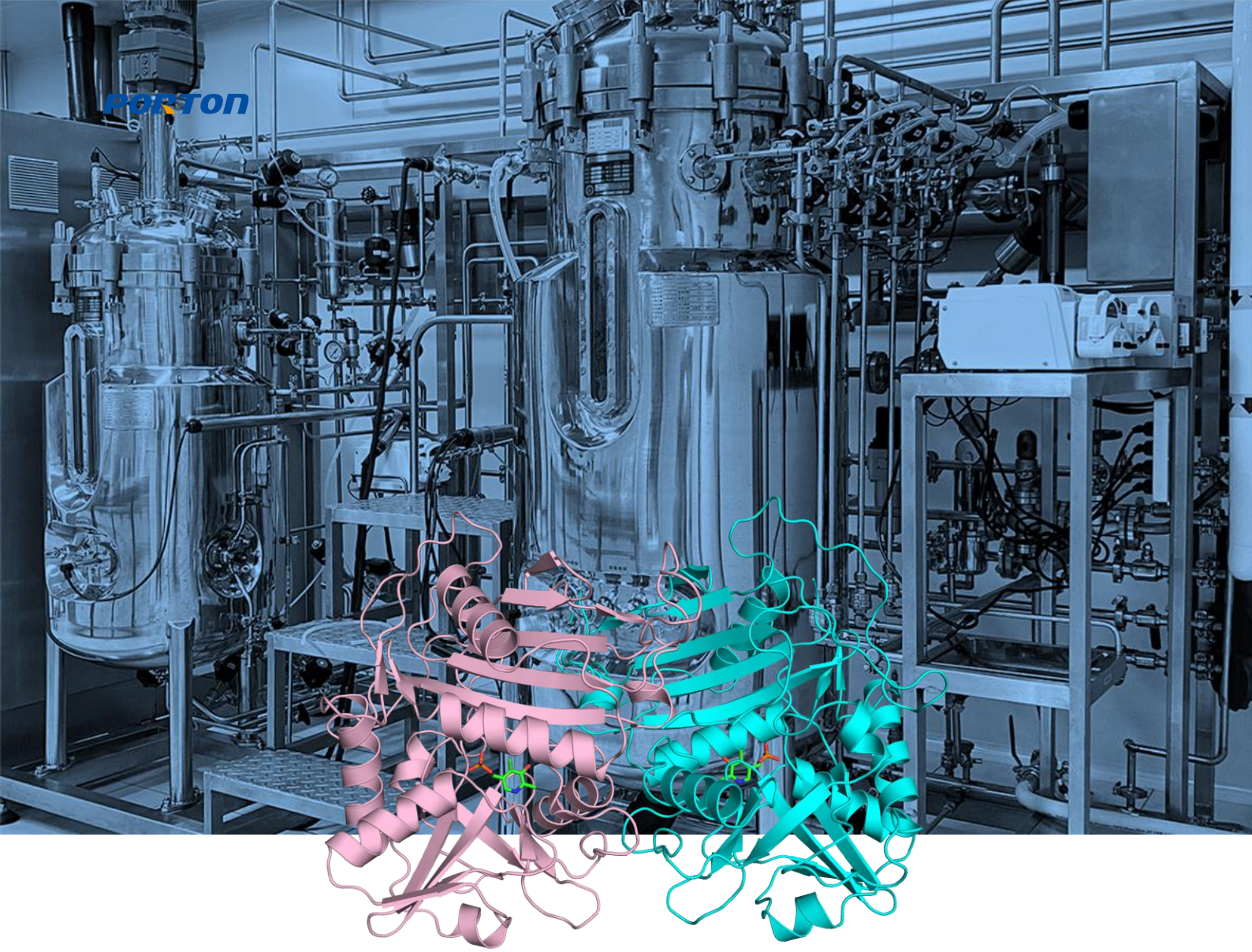


Enabling Technology

Biocatalysis:
A Powerful and Possible Greener Tool for Chemists in the Pharmaceutical Industry

Introduction

In the production of active pharmaceutical ingredients (APIs), traditional chemical synthetic methods suffer from poor selectivity, complex process (such as multiple steps, protecting and deprotecting process, chiral resolution etc.) and environmentally harmful reagents. Biocatalysis, with its potential for high efficiency, high regio-, stereo-, enantioselectivity and mild reaction conditions, has gradually become a powerful tool for chemists. Additionally, biocatalysis is also considered as “greener” technology, which is more eco-friendly, sustainable, and profitable because it is highly correlated with the 12 principles of Green Chemistry, as shown in Figure 1 below.

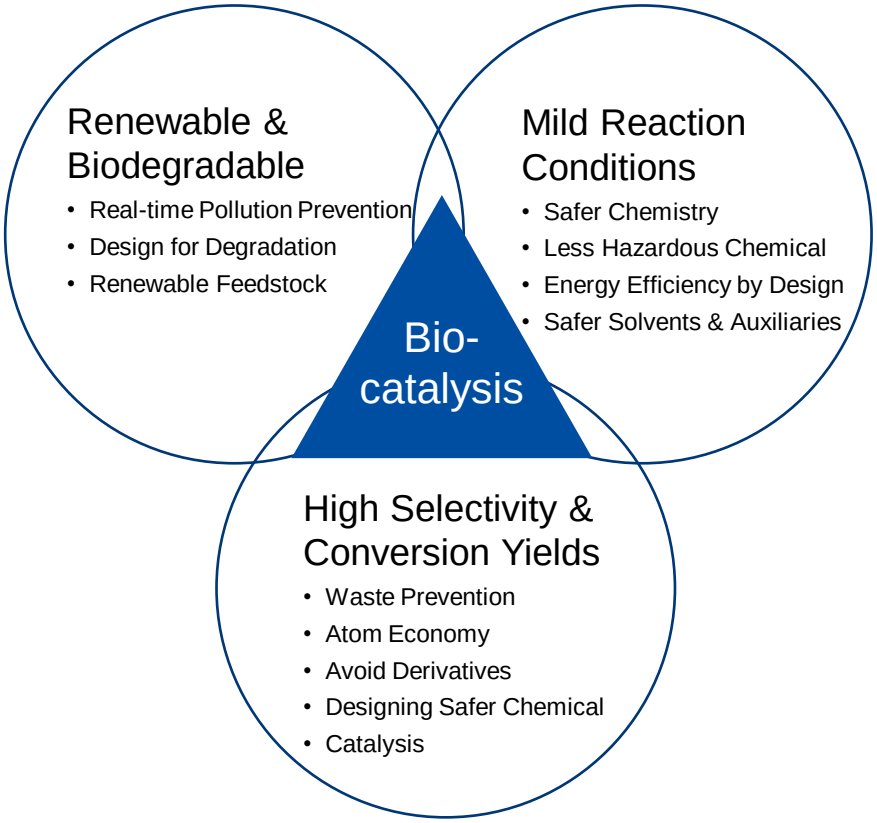


Figure 1. Correlation between green chemistry principle and enzymatic catalysis

Biocatalysis at Porton: End-to-end Enzymatic Solution Services

In an effort to uphold and practice Porton's core values of "customer first and pursuit of excellence” of Porton and meet the growing demands of customers for the greener, more efficient and economical production of pharmaceutical intermediates through biocatalysis, Porton has built full functional biocatalysis R&D labs and manufacture facilities since 2018, aiming to provide end-to-end enzymatic solutions for our customers’ projects, from enzyme/gene identification to chemical production.

Porton has built an experienced & dedicated team in R&D and manufacture providing end-to-end services, hybrid skills including Chem-enzymatic process, Multi-enzyme cascade process, Enzyme immobilization R&D and application, Flow enzymatic process with mutually beneficial flexible collaboration model including FTE services, process R&D, enzymatic chemical production, cost-driven continuous optimization to meet customer’s diverse demands at different stages (Figure 3).

Figure 2. Full function labs

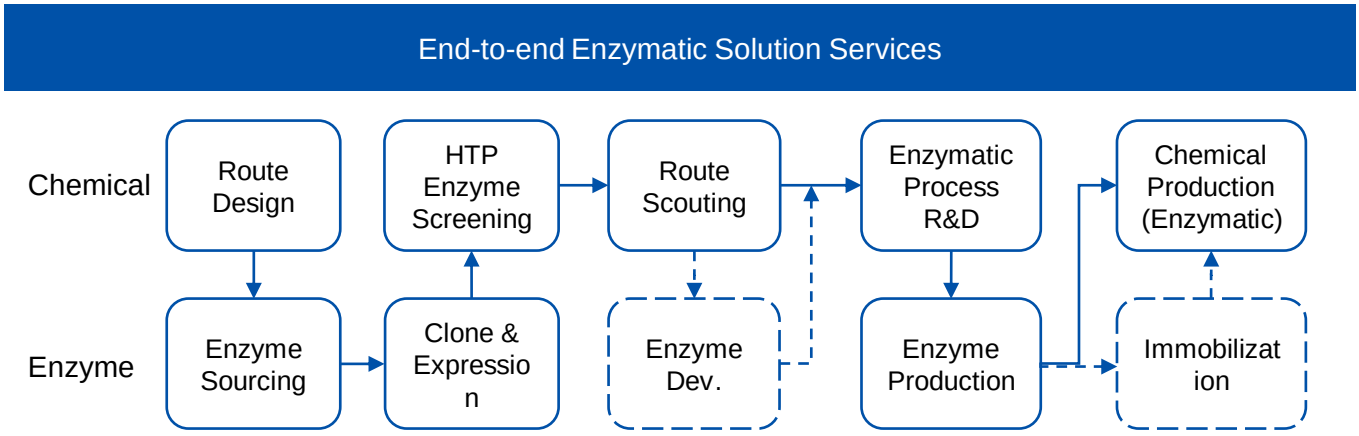


Figure 3. End-to-end enzymatic solution services

Currently, Porton has built a proprietary, free-to-operate, enzyme library, including 1500+ enzymes (covering 80+ reaction types). The majority of them are on-shelf and ready-to-use, and it keeps growing as development proceeds. To support the chemical production at different scales from mg to MT at different stages, Porton has built flexible fermentation facilities scaling from 5L to 10,000 L capable of producing enzyme (enzyme powder and cell lysate) in house from mg to MT (Figure 4).

An excellent enzyme is typically highly customized due to its substrate specificity. For a specific substrate, a vast screening is highly recommended to identify the best enzyme. At Porton, we conduct this screening in a high-throughput manner. Enzymes are typically pre-made in 96-well plate and other reaction components are transferred into each well by Multi-channel pipettes (8, 12 and 96 channels). Incubated for a short period of time, diverse and high-efficiency inspections, like colorimetric, absorbance change and also high throughput HPLC/GC in some cases, are utilized to identify the best hit based on the different characteristics of substrates, products and reaction mechanisms.

Figure 4. Fermentation facilities

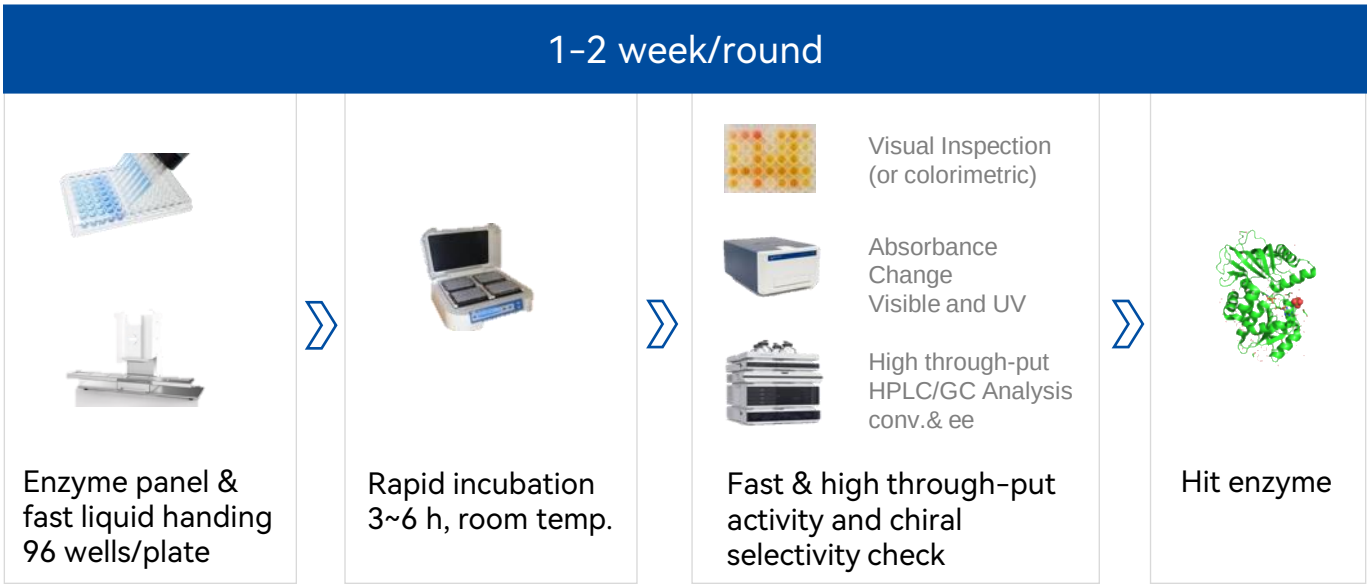


Figure 5. High throughput enzyme screening

From the quality and safety point of view, potential impurities associated with small molecule APIs manufactured using enzymes will be an issue. Although different purge and control strategies are considered to ensure these sensitive biomolecules that would not survive under standard chemical processing unit operations, such as filtration, extraction, salt formations etc. Eight commonly applied methods for protein residue analysis are summarized in below table 1.

Methods		Remarks
1	UV Absorbance	• USP method
2	Bicinchoninic Acid (BCA) Method	• USP method ○ Commercial kit available ○ Pregabalin intermediate analysis • Available in Porton
3	Bradford Method	• USP method ○ Commercial kit available ○ Pregabalin and Atorvastatin intermediate analysis • Available in Porton
4	Lowry Method	• USP method ○ Commercial kit available
5	Amino Acid Analysis	• USP method • Available in Porton
6	Polyacrylamide Gel Electrophoresis (SDS-PAGE)	• Available in Porton ○ Commercial kit available
7	NanoOrange®	○ Commercial kit available
8	Enzyme Linked Immunosorbent Assay (ELISA)	• Customized

Table 1. Methods for residual protein detection

Applied and Successful Cases

With the purpose of enabling customer's fast and greener access to key chiral building blocks, Porton biocatalysis team provides route design & route scouting, proof-of-concept study, enzyme screening, enzymatic process research & development and enzymatic product manufacture working closely with the dedicated brother teams. Porton has rich experience in using enzymatic approaches to produce chiral amines, alcohols, acids/esters/amides, UAAs, etc. Selected examples are listed in the below table 2.

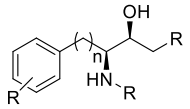
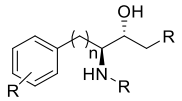
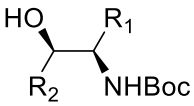
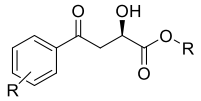
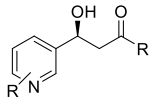
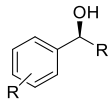
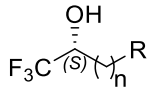
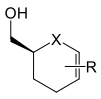
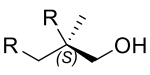
Catalogs	Product	Enzyme	Conversion	de/ ee Value	Production Scale
Chiral alcohol		Ketone reductase	99.9%	de > 99%	MTs
			≥97%	de > 99%	MTs
			DKR ≥99%	de > 98%	MTs
			≥99%	ee ≥ 99.9%	Hundreds of kg
			≥99%	ee ≥ 99.9%	Kgs
			≥99%	ee ≥ 99.9%	Multi products Hundreds of kg
			≥99%	ee > 99.9%	Dozens of kg
		Lipase	>40%	ee > 99%	Kgs
			>45%	ee > 99%	Dozens of kg

Table 2. Selected examples at Porton

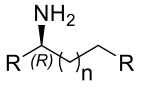
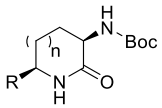
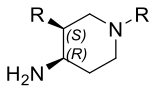
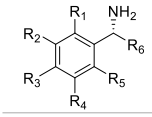
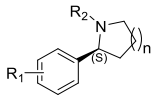
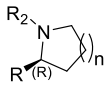
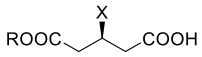
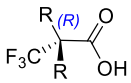
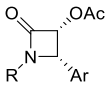
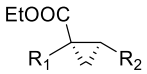
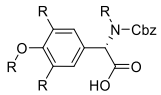
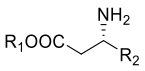
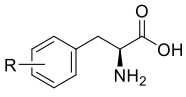
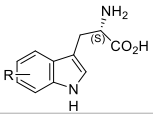
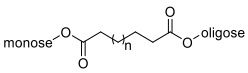
Catalogs	Product	Enzyme	Conversion Rate	de/ ee Value	Production Scale
Chiral amine		ω -transaminase	>85%	ee >99.9%	Grams
			>80%	de > 99%	Kgs
			>80%	de > 99%	Multi Products Kgs
			>85%	ee >99.9%	Multi Products Hundreds of kg
		Imine reductase	≥99%	ee ≥ 99.9%	MTs
		Monoamine oxidase	≥99%	ee ≥ 99.9%	MTs
Chiral acid		Lipase	≥98%	ee ≥ 92%	MTs
		Amidase	≥47%	ee ≥ 92%	MTs
Chiral ester		Acylase	>42%	ee > 99%	MTs
		Lipase	>44%	ee > 99%	MTs
Chiral UAA		Lipase	DKR >85%	ee > 99%	Kgs
			>95%	ee > 99%	Hundreds of kg
		α- transaminase or amino acid dehydrogenase or amino acid lyase	>95%	ee > 99%	Mutli Projects Delivered from kg to MTs
		Tryptophanase	>95%	ee > 99%	Mutli Projects Delivered from kg to MTs
Glucoside		Lipase	>85%	-	Kgs

Table 2. Selected examples at Porton

Enzyme Immobilization and Application

Despite there are much better in active pharmaceutical ingredients production, owing to much greater efficiency and far less detrimental contaminants, as compared to classical chemical synthesis, their performances are significantly impacted by their long-term instability, environmental factors and the technical challenges to recover the active enzymes for reuse. As a consequence, the production costs tend to remain a significant bottleneck if the catalysts are used as disposable reagents.

One of the most significant and broadly used techniques to overcome these limitations and make the enzyme utilization more favorable is enzyme immobilization. Porton has developed enzyme immobilization technology and implemented in commercially production, see figure 6 and 7. Using immobilized enzymes both in batch and flow (PBR) mode, enzymes can be recovered easily and reused more than 40 times, dramatically reducing both enzyme and production costs.

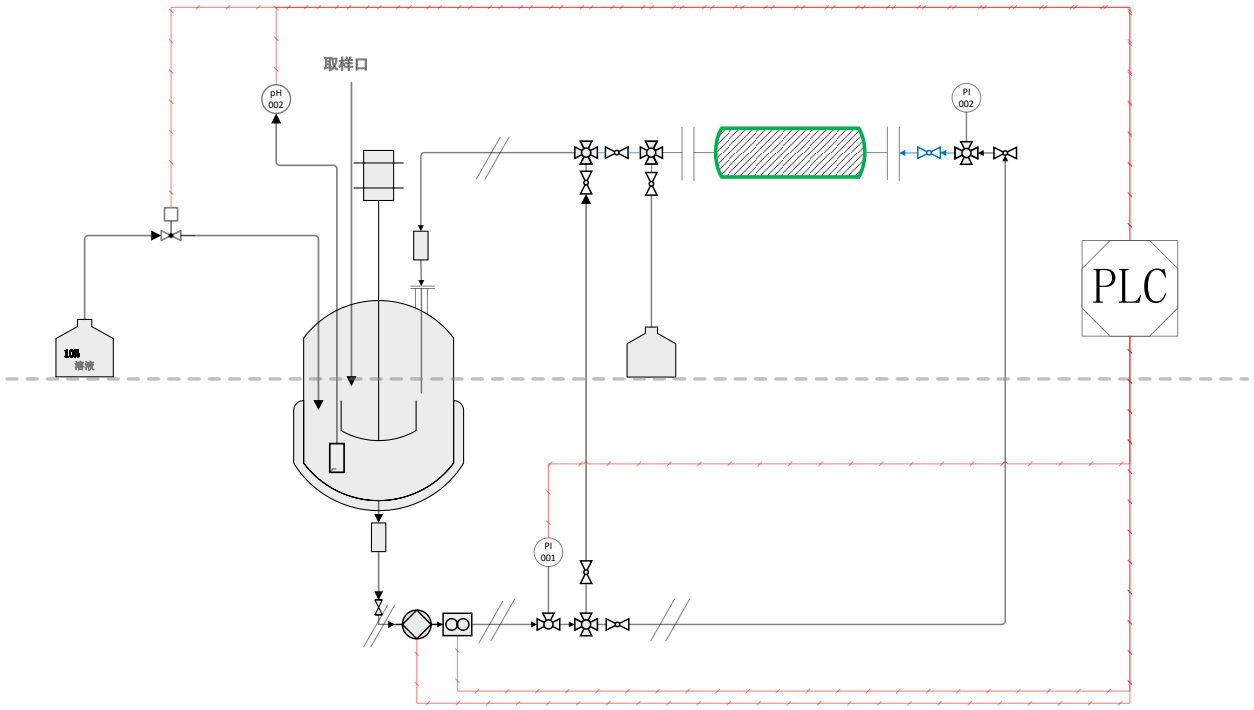


Figure 6. Flow setup for multipass experiments in a PBR

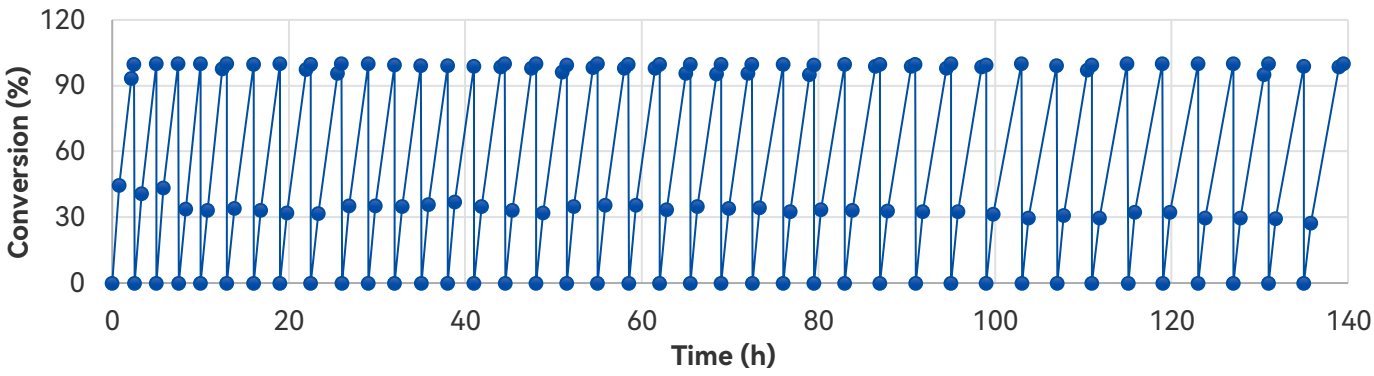


Figure 7. Batch cycles of biocatalytic process using immobilized enzyme

Conclusion

Biocatalysis is becoming increasingly popular and is a powerful, greener tool for chemists in the pharmaceutical industry, owing to its much greater efficiency, much higher selectivity and much lower cost, as well as the development of advanced technologies such as AI-aided enzyme discovery, quality- and cost-based process development, and enzyme immobilization.

Since 2018, Porton has devoted significant resources to build this technology platform, aiming to provide end-to-end enzymatic solutions to customers and great progresses are achieved. And also, the company remains committed to continuous development of this technology to enable public's fast and greener access to good medicine.

About Porton Biocatalysis Technology Platform

Porton Biocatalysis Technology Platform, driven by an experienced, dedicated biocatalysis team and advanced equipment, focuses on building a comprehensive biocatalysis platform and capabilities. We provide clients with more efficient, more eco-friendly biocatalysis solutions and "end-to-end" CDMO services including enzyme development, enzyme preparation, biocatalytic process development and product production. This helps accelerate the commercialization process of new drugs, enabling the public's early access to good medicines.

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