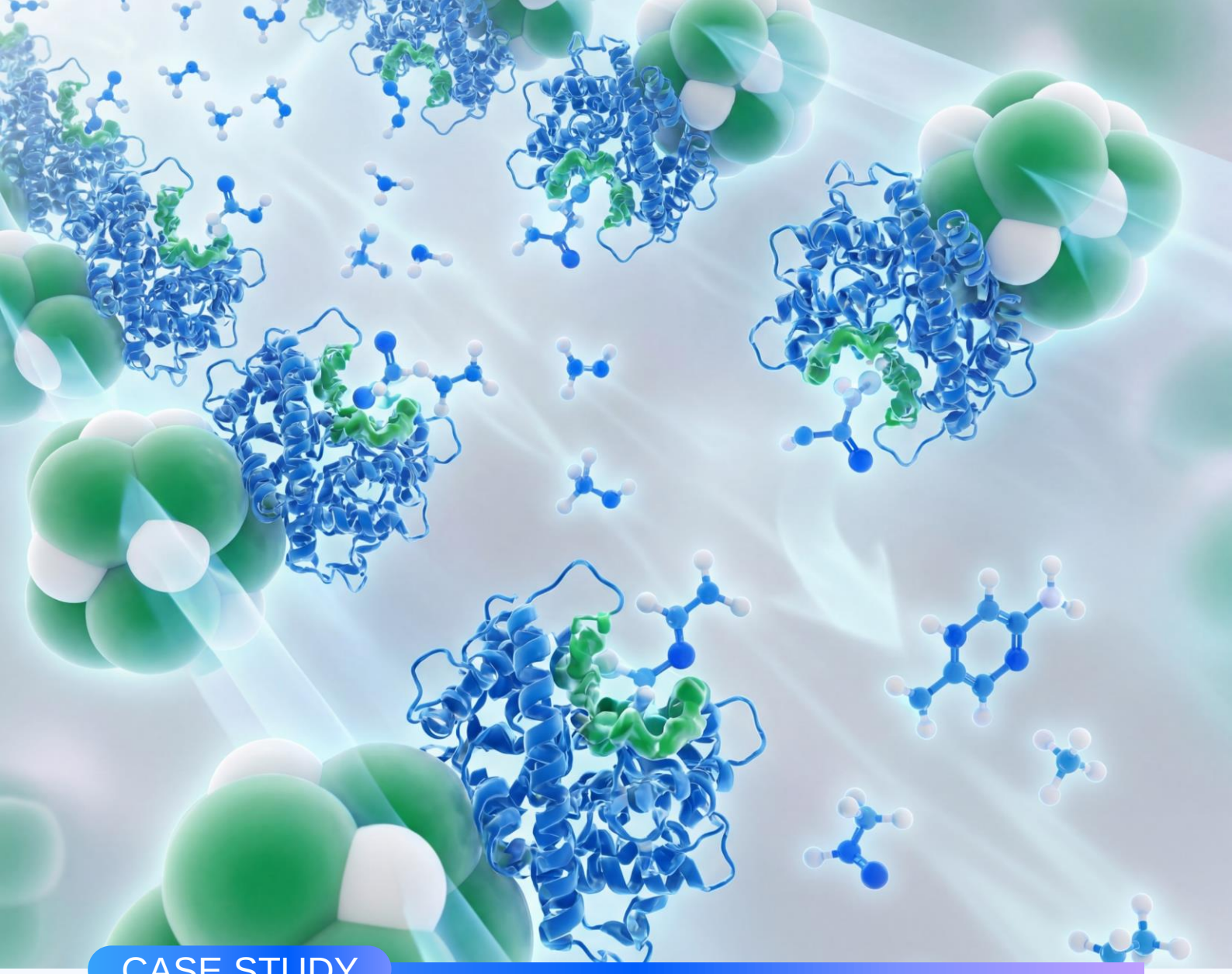


CASE STUDY

The Perfect Integration of Immobilized Enzymes and Flow Chemistry: A Powerful Booster for the Commercialization of Biocatalysis Technology

PORTON

In traditional organic chemical synthesis, issues such as poor selectivity, harsh reaction conditions, and environmental pollution are commonly encountered. In contrast, Biocatalysis is regarded as a "greener" technology due to its high efficiency, excellent regio-/stereo-/enantioselectivity, and mild reaction conditions, offering environmental sustainability and economic benefits. In the fine chemical industry, companies are actively investing in this capability to enhance the atom economy of synthetic processes, reduce waste generation, improve ESG ratings, and strengthen sustainable competitiveness.

Since 2018, Porton has been building its biocatalysis capabilities. Through ongoing investments, the company has progressively established fully functional laboratories and production facilities, offering end-to-end biocatalysis solutions, including enzyme/gene mining, sourcing, screening, and development, enzymatic process development, enzyme fermentation production, as well as the development and utilization of immobilized enzymes. Porton has built an integrated biocatalysis delivery platform, from R&D to commercial production, aims to delivering more environmentally friendly and cost-effective biocatalysis solutions, accelerating the development and production processes of clients' drugs, enabling the public's early access to good medicines.

Critical Challenges in Enzyme Application

Despite its numerous advantages, biocatalysis still faces several challenges in practical applications, such as:

1. Free enzymes are typically single-use and non-recoverable, leading to higher costs;
2. The downstream processing steps are prone to emulsification, requiring additional defoaming and demulsification procedures, along with prolonged settling for phase separation;
3. Protein residue may remain in the chemical products, posing challenges to product quality and safety.

Immobilized Enzyme

The use of immobilized enzyme can effectively address the aforementioned issues, significantly enhancing the feasibility of applying biocatalysis technology in the synthesis of active pharmaceutical ingredients (APIs). Immobilized enzymes can be obtained by confining or attaching soluble free enzyme to solid support (e.g., polymers, inorganic materials, or membranes) through physical, chemical, or biological methods, while retaining their catalytic activity and stability. Compared to free enzyme:

1. Higher stability. Immobilized enzyme exhibits higher stability and can tolerate higher substrate concentration, temperature, and solvent ratio.
2. Reusable. Immobilized enzyme can be easily separated from the reaction mixture after the reaction, and reused in a new batch, significantly reducing the cost of enzyme.
3. Simple downstream processing. Since the enzyme protein is immobilized on an insoluble carrier, procedures for protein denaturation or enzyme removal are eliminated. Immobilized enzyme can be recovered simply by filtration.
4. Less protein residual. Emulsification can be avoided during product separation and significantly reduces enzyme protein residues in the final product.

Reactor and Operation Mode of Immobilized Enzyme

The reactor types and operation modes of immobilized enzyme are diverse (Figure 1), encompassing the traditional batch stirred tank reactor (BSTR), continuous flow stirred tank reactor (CSTR), rotating packed bed reactors (RPBR), fluidized bed reactors (FBR), packed bed reactor (PBR), and recycle reactor (RCR).

Traditional stirred-tank reactors (Figures 1a and 1b) rely on mechanical agitation to suspend immobilized enzyme particles, offering high mass transfer coefficient and convenient temperature control. They can be operated in batch or fed-batch mode, making them suitable for systems with low substrate/product inhibition and f requiring frequent pH/temperature adjustments, especially for lab scale trials and multi-product alternating production. However, in such reactor, immobilized enzyme particles are prone to being shattered by the mechanical stirrer, leading to inactivation.

Rotating packed bed reactors (Figure 1c) can address the aforementioned issues. By confining immobilized enzymes into a cage-like structure fixed to the stirrer shaft, acting as an agitator during high-speed rotation. This reactor offers high mass transfer efficiency and is suitable for reaction systems with high viscosity or poor substrate solubility. However, scaling up production or switching between batches requires disassembly and modification of the reactor, making the process more complex and time-consuming.

In fluidized bed reactors (Figure 1d), the reaction mixture flows from bottom to top, suspending carrier particles in the fluid to achieve efficient contact. However, flow velocity must be controlled to prevent enzyme loss.

Packed bed reactors (Figures 1e and 1f), by contrast, represent a combination of immobilized enzymes and continuous flow, integrating the high activity of enzyme, the recoverability and stability of immobilized enzyme, and the engineering advantages of continuous production, enabling significant improvements in space-time yield (STY), enhanced mass transfer, reduced inhibition, and elimination of additional catalyst separation steps.

Compared to traditional stirred tank reactor, packed bed reactor exhibits smaller scale-up effects, allowing for simple and rapid scale-up of the system. And, it is also easier to operate and control than rotating packed bed reactor, and fluidized bed reactor, requiring no reactor modifications or concerns about enzyme loss. Fixed-bed reactors represent the perfect integration of biocatalysis and flow chemistry technologies, making them an ideal solution for the industrial application of immobilized enzymes.

When using a packed bed reactor, the reaction solution is pumped into the reactor, and the residual time per cycle is controlled by adjusting the flow rate. The operation mode can be categorized into one-pass mode (Figure 1e) and recycle mode (Figure 1f). In one-pass mode, the reaction is completed as soon as the reaction solution exits the packed bed reactor. In recycle mode, the reaction solution exited from the reactor is pumped back into the packed bed for further reaction until completion.

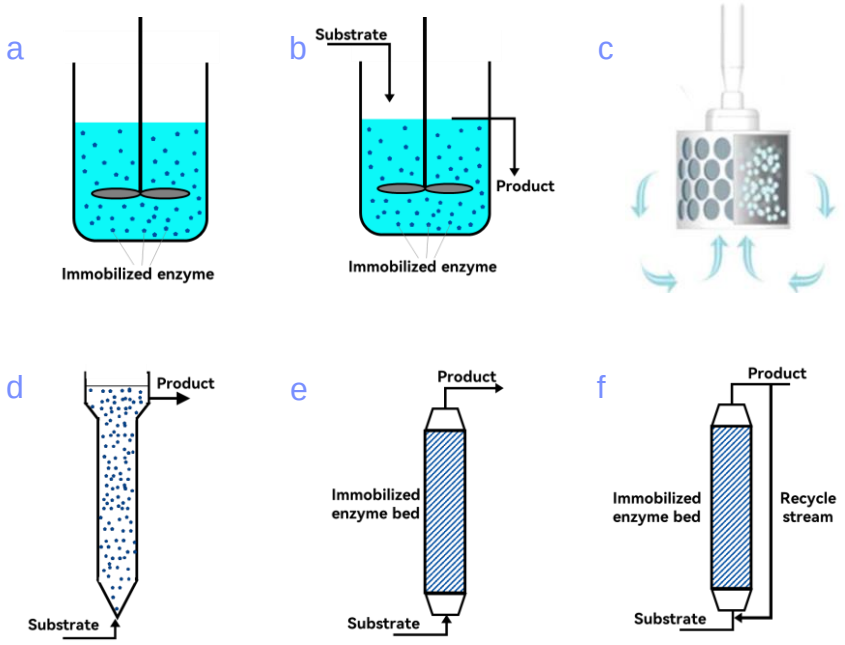


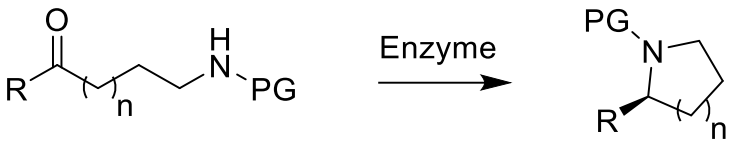
Figure 1. Common types of immobilized enzyme reactors: (a) Batch Stirred Tank Reactors; (b) Continuous Flow Stirred Tank Reactor (CSTR); (c) Rotating Packed Bed Reactor (RPBR); (d) Packed Bed Reactors (FBR); (e, f) Packed Bed Reactors (PBR).

Case Study

Adhering to Porton's core value of "Pursuing Excellence," Porton biocatalysis team has designated "enzyme immobilization technology and its industrialization" as a core strategic technology. With sustained resource investment, the team has systematically established a platform through a 3-step approach: carrier screening, immobilized enzyme process development, and immobilized enzyme application. This has enabled the successful immobilization and application of multiple enzymes.

In a project of biocatalytic synthesis of a chiral amine, collaborated with the chemistry team, Porton biocatalysis team developed a free enzyme process in 2020 and has implemented to commercial production. With the intensification of competition, the cost advantage of route is gradually diminishing.

An immobilized enzyme processes is developed by biocatalysis team. By co-immobilizing imine reductase (IRED) and glucose dehydrogenase (GDH) on a polymer carrier, they successfully prepared a dual-enzyme cascade "co-immobilized microsphere". This co-immobilized microsphere was then packed into a packed bed reactor for continuous flow reactions. The immobilized enzyme can be recovered and reused for more than 40 times and give a Space-time yield (STY) of 1090 $g \cdot L^{-1} \cdot d^{-1}$. Recently, the process is successfully scaled up by 1700-fold in pilot plant (Figure 2), the process is run for almost a month and the immobilized enzyme can be recovered and reused for more than 37 times without significant activity loss (terminated due to regular site maintenance). These results conclusively demonstrate the feasibility and scalability of this process, laying a solid foundation for achieving efficient, reproducible, and large-scale production in the future.



Scheme 1: enzymatic process of chiral amine synthesis

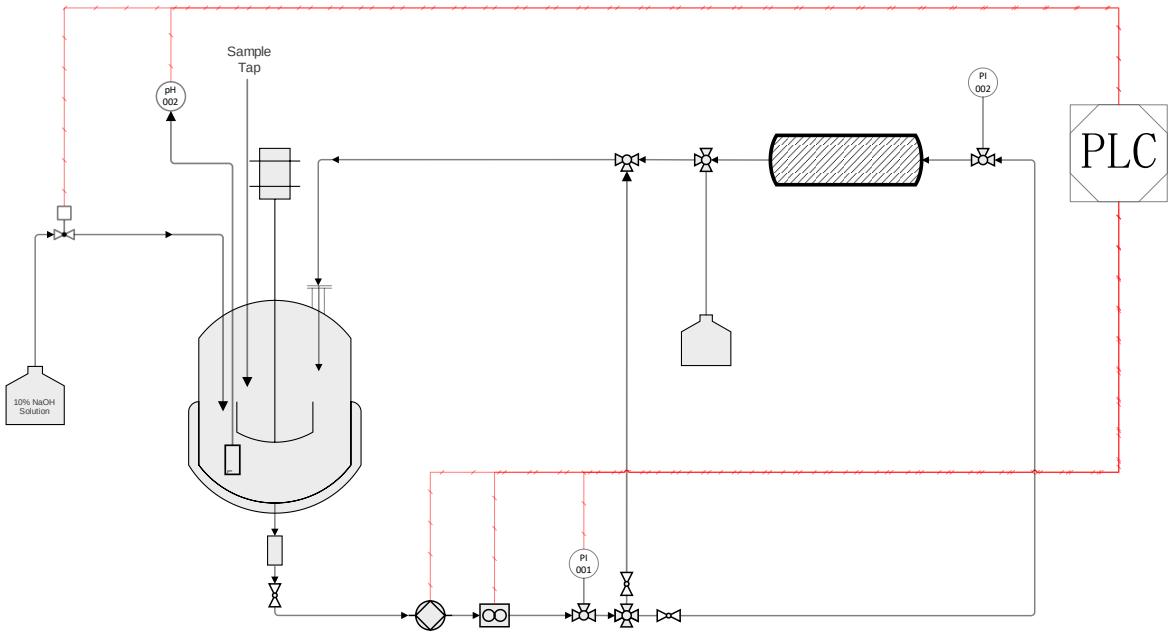


Figure 2. Schematic Diagram of Co-Immobilized Enzyme Continuous Reaction Equipment in Pilot Plant

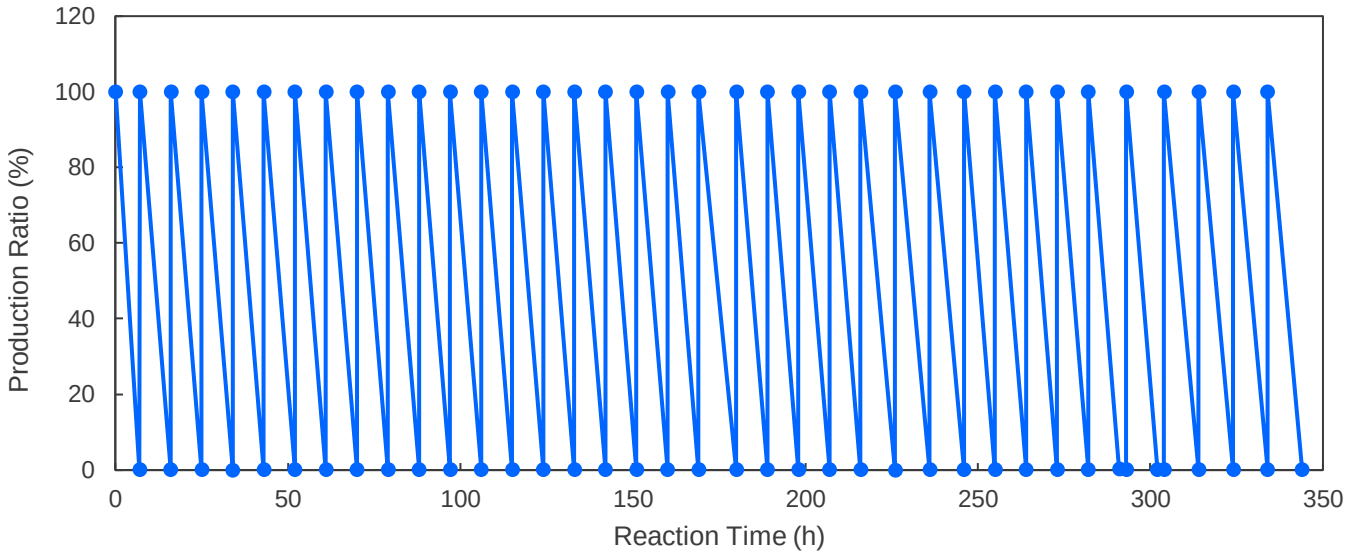


Figure 3. The result of continue flow reaction of co-immobilized enzyme.

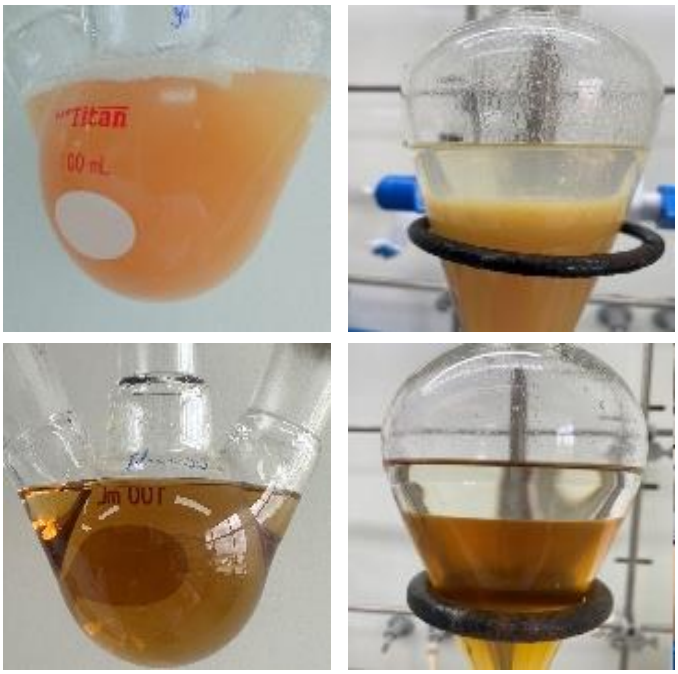


Figure 4. Comparation of reaction slurry and extraction: free enzyme process (upper), immobilized enzyme process (lower).

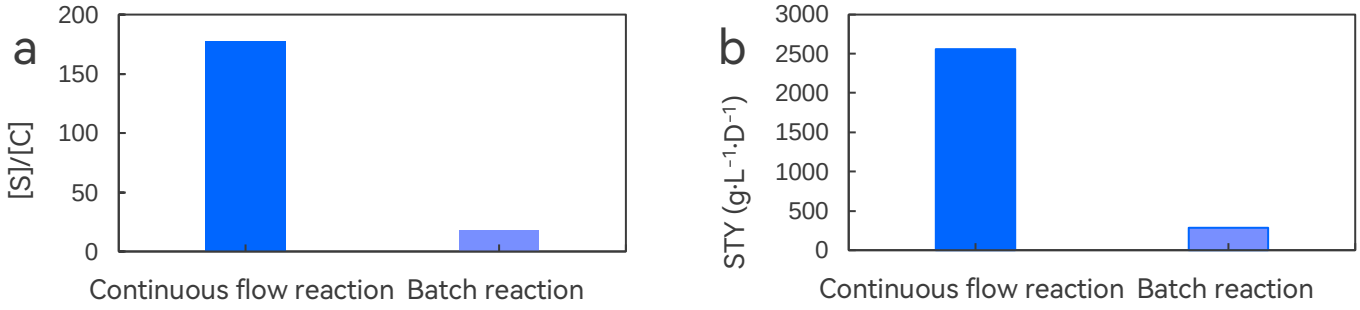


Figure 5. Comparation of parameters between free enzyme process and immobilized enzyme process: [S]/[C] (a), STY (b). [S]/[C] represents the mass ratio of substrate to catalyst, and STY refers to space-time yield (STY), defined as the amount of target product generated per unit time and per unit volume.

Compared to the free enzyme process, this technology eliminates the need for enzyme charging in each batch reaction and avoids emulsification during downstream processing (Figure 4). It requires no enzyme denaturation or demulsification steps, significantly reducing extraction and phase separation time. Additionally, due to enzyme reuse, the total enzyme consumption is drastically reduced by ~90%, achieving an S/C ratio of 177 (Figure 5a)—10 times higher than that of free enzyme systems. The overall material cost is reduced by >20%. Coupled with continuous-flow technology, the reaction volume is minimized, and the space-time yield (STY) increases to 2560 $g \cdot L^{-1} \cdot D^{-1}$ (Figure 5b), truly realizing green and efficient manufacturing with " small space, high capacity."

In another project of biocatalytic synthesis of a chiral amine, similar strategy was employed. Lab-scale experiments demonstrated that immobilized enzyme can be reused for 40 time, with the S/C ratio enhanced by 18.5-fold relative to the free enzyme system. Additionally, the STY was 289.7-fold higher than that of the batch reactor process. Relevant progress has been published in Organic Process Research & Development, DOI: 10.1021/acs.oprd.4c00130.

Furthermore, the Porton biocatalysis team has also developed a range of high-activity, high-stability immobilized enzymes, including ketoreductase (KRED, Figure 6) and transaminase (ATA, Figure 7), and implements to lab and industrial scale successfully.

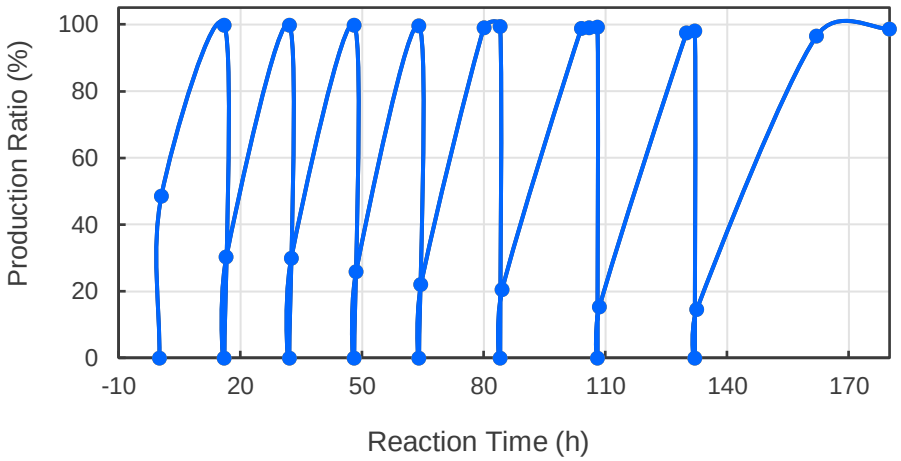


Figure 6. Reaction of immobilized ketoreductase.

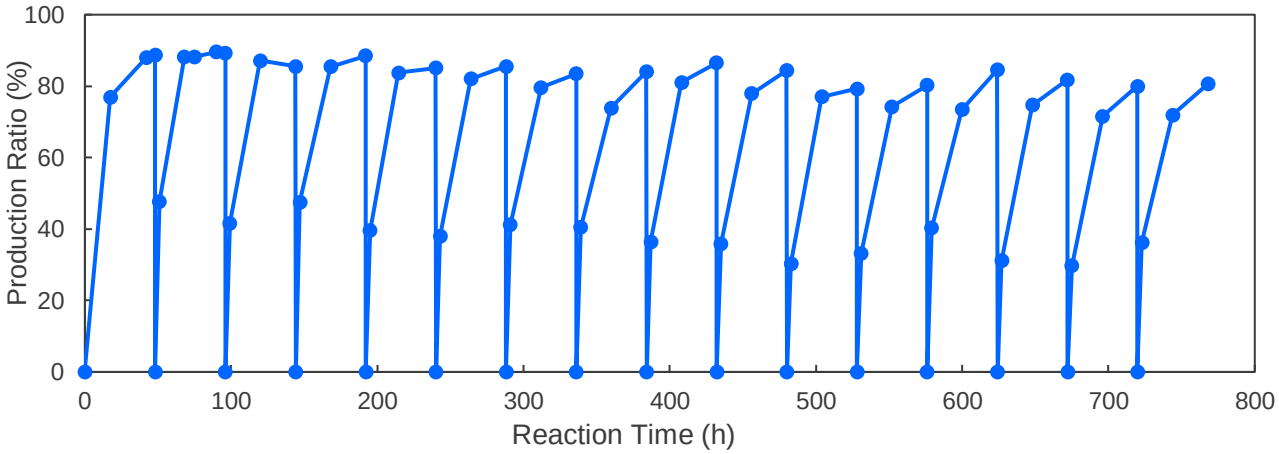


Figure 7. Reaction of immobilized transaminase.

With years of dedicated research and development in enzyme immobilization technology and its industrial application, the Porton Biocatalysis team has built up extensive expertise across carrier screening and modification, immobilized enzyme process development and optimization, and commercial scale-up implementation of immobilized enzymes.

In the future, we will continue to focus on driving the R&D and commercialization of diverse immobilized enzymes in categories and formulations, and further expand the application of biocatalysis technology within the pharmaceutical industry.

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 earlier access to good medicines.

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