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REVIEW ARTICLE

Harnessing Biocatalysis for Green Chemistry: Emerging Innovations and Sustainable Frontiers

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ABSTRACT

Biocatalysis, which combines green chemistry and white biotechnology, provides a sustainable alternative to conventional chemical synthesis. Enzymes and microbial systems act as selective, renewable catalysts, enabling complex reactions under mild conditions with minimal waste and high atom economy. In pharmaceuticals and fine chemicals, biocatalysis supports shorter synthetic routes, improved stereoselectivity, and regulatory compliance. Advances in enzyme engineering, such as directed evolution, rational design, and machine learning, have expanded the capabilities and robustness of biocatalysts. Techniques such as enzyme immobilization and whole-cell catalysis enhance stability and cost efficiency. Case studies, including simvastatin and pregabalin synthesis, showcase its practical benefits. The integration of chemoenzymatic strategies merges enzymatic precision with chemical versatility, streamlining synthesis. Aligned with the 12 principles of green chemistry, biocatalysis reduces hazardous reagents, lowers energy use, and enables renewable feedstocks. As regulatory bodies promote greener practices, its industrial adoption grows. Future directions include AI-guided enzyme discovery and continuous flow systems for scalable, eco-friendly production. This review explores the current landscape, key innovations, and future directions of biocatalysis in sustainable chemical manufacturing.

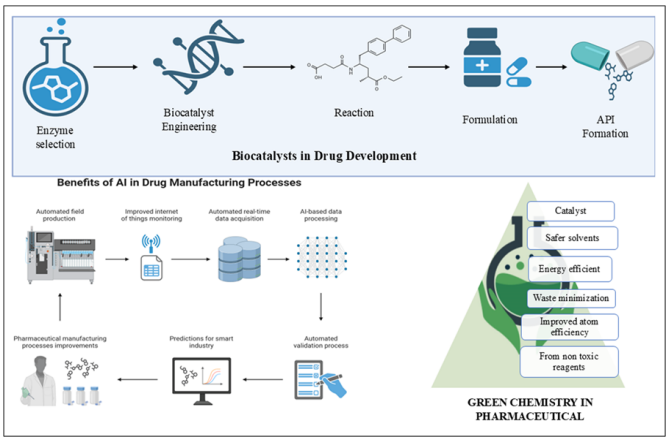
Keywords: Biocatalysis; Green Chemistry, White Biotechnology, Enzyme Engineering, Sustainable Synthesis, Biotransformation, Eco-friendly Catalysis

HIGHLIGHTS

- This review positions biocatalysis as a key role tool for environmentally responsible synthesis, aligning with green chemistry principles through reduced water, mild conditions, and enhanced atom economy.
- It classifies various biocatalyst systems, including isolated enzymes and microbial cells, and evaluates recent advances in enzyme engineering, such as immobilization techniques and precision design.
- The paper presents industrially relevant case examples, including pregabalin, to demonstrate how chemoenzymatic methods enhance selectivity, yield, and process sustainability in pharmaceutical manufacturing.
- It surveys transformative innovations like artificial intelligence-driven enzyme design, nanoscale biocatalysts, and enzymatic cascades that are redefining the boundaries of drug synthesis.
- Current limitations in industrial biocatalysis are addressed, alongside prospective solutions such as

synthetic biology integration, metagenomic exploration, and continuous flow process development.

Graphical Abstract



Overview of Biocatalysts in Pharmaceuticals

INTRODUCTION

Biocatalysis has emerged as a key approach in sustainable chemical synthesis, especially within pharmaceutical and fine chemical industries. It utilizes enzymes or whole-cell systems to carry out chemical transformations under environmentally benign conditions, often in water and at ambient temperature and pressure[1]. These biological catalysts, derived from renewable resources, offer high selectivity, chemo-, regio-, and stereoselectivity, making them suitable for complex molecular modifications without the need for protecting groups or multiple synthetic steps[2]. This efficiency leads to significant reductions in energy consumption, byproduct formation, and overall waste, aligning well with the objectives of green chemistry. Common sustainability metrics such as the E-factor and atom economy consistently reflect the advantages of biocatalytic processes in terms of reduced environmental burden and enhanced resource utilization[3].

Biotechnological advancements have greatly broadened the scope of applicable enzymes. The availability of genome databases has facilitated the identification of novel biocatalysts. Techniques such as directed evolution, gene shuffling, and recombinant expression have enabled the rapid enhancement of enzyme properties, including specificity, stability, and activity. Cost-effective enzyme immobilization methods further support operational efficiency by enhancing catalyst longevity and reusability[4]. Recent progress in computational biology and bioinformatics has enabled the identification of biosynthetic gene clusters involved in the production of complex natural products[5]. This not only improves access to biologically active scaffolds but also allows for their tailored modification through engineered enzymes. Such advances position biocatalysis as a central technology in the development of cleaner, more efficient, and scalable synthesis pathways[6]. As environmental regulations and sustainability goals become increasingly prominent in pharmaceutical manufacturing, biocatalysis offers a forward-looking solution that integrates the benefits of biotechnology with the demands of modern green chemistry[7, 8].

THE FUNDAMENTALS OF BIOCATALYSIS

Biocatalysis has become integral to pharmaceutical synthesis, offering selective, efficient, and environmentally favorable alternatives to conventional chemical methods. Biocatalysts operate under mild conditions, often in aqueous media, reducing energy consumption and waste. The main types of biocatalysis used in pharmaceutical applications include whole-cell catalysts, immobilized enzymes, microbial consortia, and engineered systems, are summarized in Table. 1 [9].

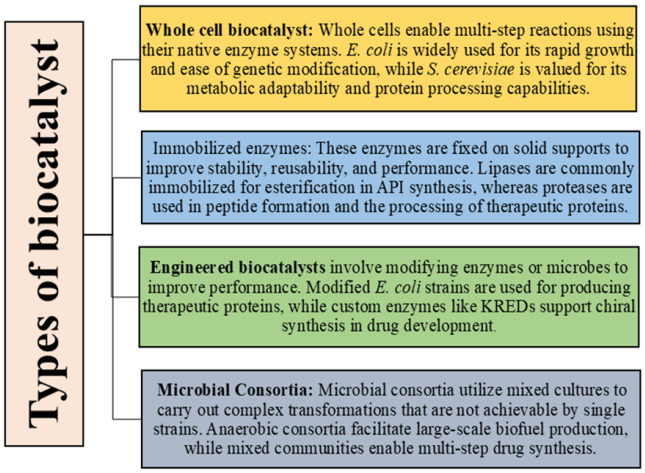


Fig. 1: Types of biocatalysts

The Role of Enzymes as Green Catalysis:

Enzymes, as particular biological catalysts, play a critical role in pharmaceutical synthesis. Their ability to accelerate reactions with remarkable selectivity and under mild conditions makes them essential in the manufacturing of numerous active pharmaceutical ingredients as shown in Table. 1 [10].

Advantages and Challenges in Industrial Use:

Compared to traditional chemical methods, biocatalysis provides notable benefits, especially regarding efficiency and environmental sustainability. By using natural enzymes, biocatalysis typically results in more environmentally friendly and cost-effective processes[13]. Unlike traditional chemical methods, biocatalysis operates under milder conditions, reducing energy consumption and waste generation. It also provides enhanced selectivity and yields,

contributing to reduced by-products and fewer purification steps. However, conventional chemical synthesis remains essential in certain industrial applications where biocatalysis may not be feasible or cost-effective. Therefore, integrating both approaches can offer a balanced and optimized strategy for chemical production, leveraging the strengths of each method to address a broader range of manufacturing challenges[14].

Table 1: Classification of Biocatalyst

Biocatalyst type	Enzyme class	Example enzymes	Functions in drug synthesis	Cofactor requirements
Oxidoreductase	EC 1	Alcohol dehydrogenase, monoxygenases	Oxidation/ reduction steps	NAD+/NADP+, FAD, heme
Transferases	EC2	Transaminases, methyltransferases	Functional group transfer	PYRIDOXAL PHOSPHATE, SAM
Hydrolases	EC 3	Lipases, esterases, proteases	Bond cleavage Reactions	Metal ions (e.g. Zn^{2+})
Lyases	EC 4	Aldolases, decarboxylases	Carbon-carbon bond formation	ThDP, PLP
Isomerases	EC 5	Epimerases, racemases	Stereoselective bond formations	NAD^+, metal ions
Ligases	EC 6	Synthetases, DNA ligases.	Bond formation with ATP	ATP, CoA
Translocases	EC 7	ABC transporter, MATE transporter, secyeg, Tat system	Transport of substances across the membrane	ATP, PMF, Na^+/H^+ gradient

ENZYME ENGINEERING AND DISCOVERY STRATEGIES

Biocatalysis offers an environmentally friendly approach to producing fine chemicals. Enzyme engineering plays a crucial role in tailoring enzymes for optimal performance in industrial processes, enhancing both efficiency and sustainability. Recent advances in protein engineering have enabled a new paradigm: conceptually designing the desired chemical process and then adapting the biocatalysis to the required reaction conditions[44]. Protein engineering technologies play a crucial role in modifying and optimizing the traits of proteins for various applications, including enhancing their performance in chemical reactions[45].

- **Enzyme Engineering: Principles and Evolution**

Enzyme engineering involves the design and modification of enzymes to improve their performance for specific industrial and research applications. This includes enhancing properties such as catalytic activity, substrate specificity, and stability under harsh conditions[46]. Common strategies include site-directed mutagenesis, directed evolution, and immobilization to generate robust and efficient biocatalysts. Engineered enzymes are increasingly used in sectors such as biofuels, pharmaceuticals, and food technology, where they enable reactions under extreme conditions, such as high temperatures or acidic environments conditions often encountered in biomass pretreatment or chemical synthesis[47]. Hence, enzyme engineering is a dynamic and evolving field that plays a crucial role in enhancing biocatalysis for industrial applications, with a rich historical background that has shaped its current methodologies and significance[48].

Recent advancements in enzyme engineering, which utilize machine learning and computational techniques, have become essential tools in modern enzyme engineering. These methods enable the prediction of protein folding, enhance enzyme properties such as solubility and stability, and assist in determining substrate preferences[49]. Computational tools using molecular modeling, docking analyses, and molecular dynamics offer insights into enzyme-substrate interactions, structural flexibility, and binding performance. Additionally, QSAR models establish relationships between molecular structures and enzymatic activity, collectively accelerating the creation of specialized biocatalysts for use in pharmaceuticals and environmentally friendly chemical processes[50].

- **Protein engineering approaches**

Here are the most prevalent methods for protein engineering

1. Rational design
2. de novo design
3. Directed evolution
4. Semi-rational design.

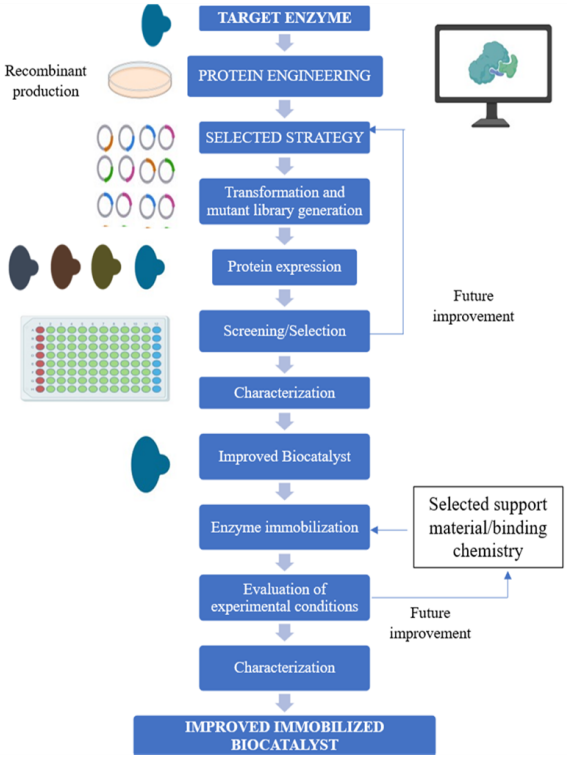


Fig. 2: Throughout the process, collaboration between computational modeling, molecular biology, biochemistry, and biophysical techniques is essential for successful protein engineering

Rational Design:

The rational design approach relies on well-defined knowledge of the protein's 3D structure and function that incorporates precise, scientific properties, such as substrate specificity, pH, or temperature stability, into the protein. In rational protein design, the initial step involves selecting a protein scaffold. This choice is informed by existing knowledge of the protein structure of a similar protein [homologous]. The subsequent decisions about which residues or regions to alter in the protein scaffold are determined by considering both the current function of the protein and the desired function after the rational design[57]. The modifications typically include making changes to the native protein sequence, such as introducing mutations, deletions, or insertions of specific amino acids. This approach aims to strategically enhance the protein characteristics based on a thorough understanding of its structure and functions as detailed in Fig. 3 of the workflow of Rational Evolution[58].

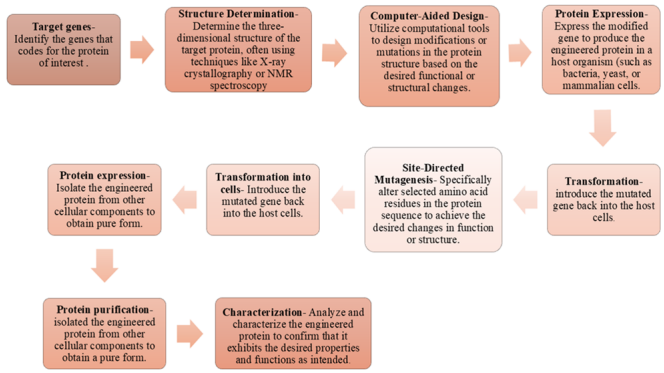


Fig. 3: The workflow of rational design

The rational design of proteins involves leveraging structural information obtained from techniques like [NMR] Nuclear magnetic resonance, X-ray crystallography, and electron microscopy. Computational protein design methods are practical tools for identifying amino acid sequences that enhance protein stability, modify binding specificity, or increase protein solubility[59].

In rational protein design, the sequence–structure–function relationship is key. This method involves identifying target amino acid residues for mutation, commonly using site-directed mutagenesis, followed by experimental evaluation of the altered proteins. However, this approach is often limited by challenges such as poor expression levels, reduced stability, or failure to achieve desired functionality. Additionally, applying rational design broadly is difficult when structural or functional data for the protein are lacking.

Directed evolution offers a powerful alternative. Unlike rational design, it does not require detailed knowledge of a protein's structure or mechanism. Instead, it relies on generating a library of random variants and selecting those with improved traits through iterative screening. This

flexibility makes directed evolution particularly valuable for enhancing protein stability, expression, and catalytic efficiency, especially when little prior information exists. As such, it is an effective tool for overcoming limitations commonly faced in protein engineering[4].

Rational Design of Cytochrome P450 Variants:

The rational engineering of cytochrome P450 variants has significantly advanced drug synthesis by enhancing catalytic efficiency and substrate specificity. These engineered enzymes enabled the streamlined production of structurally complex pharmaceutical compounds that are often difficult to synthesize using conventional chemical approaches. Their high selectivity also minimized by-product formation, reducing downstream purification steps and contributing to more cost-effective and environmentally sustainable manufacturing processes[60]. Further examples of these applications are provided in Table. 2.

Computational Approaches in Rational Design:

The integration of computational methods into rational design has further amplified these benefits. By using in silico tools to identify and introduce targeted mutations, researchers achieved substantial improvements in enzyme activity and stability. This strategy exemplifies the potential of computationally guided enzyme engineering to optimize biocatalysts for specific pharmaceutical applications. Such advances highlight the growing role of computational protein design in enabling sustainable, scalable, and efficient drug manufacturing[61].

Table 2: Rational Design in Enzyme Engineering for Drug Development

Drug Name	Target enzyme	Mechanism of Action	Rational design approach	Reference
MK-7845	SARS-COV-2 Main protease [3CLpro]	Reversible covalent inhibition via a difluorobutyl-glutamine mimic, targeting	Structural optimization to mimic glutamine cleavage site, enhancing specificity.	62
Zelenirstat	N-Myristoyltransferases [NMT1/2]	Dual inhibition disrupts protein myristoylation, affecting cell signaling and energy production.	Targeted design to inhibit myristoylation, impairing viral replication and tumor growth.	63
Vafidemstat	Lysine-specific demethylase 1 [LSD1] and monoamine oxidase	Dual inhibition modulating gene expression and neurotransmitter levels	Rational design to inhibit both enzymes, addressing neuropsychiatric disorders	64

Directed Evolution [D.E.]: D.E. is an effective technique for generating proteins with particular functions. Directed evolution produces random mutations in the gene of interest while requiring no protein structure knowledge. Instead, random mutations are introduced into the target gene to generate variants with desirable traits. The directed evolution method refers to a method that mimics natural evolution to enhance proteins. This entails rigorous selection and screening techniques that identify proteins with optimum functionalities, such as enhanced diversity in genes, improved binding, catalytic properties, and increased stability in varying temperatures and environments. This technique is valuable for developing proteins with specific medical applications, like drug delivery or treatments[65].

The directed evolution comprises repetitive cycles involving (a) introducing variations in a specific gene sequence through random mutagenesis and/or DNA recombination. (b) identifying the preferred protein variants through high-throughput screening or selection (c) amplifying the selected genes to further analysis. The substantial advancements in library creation techniques of genetic material, enhancing screening capabilities, refining DNA synthesis methods, and computational approaches are the foundational technologies underlying the remarkable achievements, allowing for precise customization of biocatalysis to meet specific requirements. This cyclical process is schematically represented in an accompanying Fig. 5 [66].

A green alternative- Directed evolution presents a sustainable solution by conferring unique specificity to enzymes, facilitating environmentally friendly biocatalysis. Enzymes, shaped through this evolutionary process, supplant harsh industrial methods with gentler biotechnology, eliminating the need for toxic metals displaced chemical catalysis, particularly in asymmetric synthesis, resulting in reduced organic solvent usage, diminished side product formation, and minimized waste production. This exemplifies a significant stride toward greener alternatives in industrial processes.

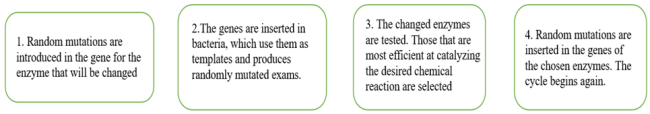


Fig. 4: Workflow of directed evolution of enzymes

A directed evolution experiment has three critical steps: Mutagenesis, Screening, and Gene Amplification.

- **Generation of mutant protein library:**

In the process of generating a mutant protein library, it is imperative to articulate a precise goal in protein engineering

that aims at achieving functional diversification or substrate specificity. Employed targeted libraries characterized by a favorable balance of advantageous versus detrimental mutations serve as a strategic initial approach. Key methodologies, such as error-prone Polymerase Chain Reaction (PCR) and DNA shuffling, are frequently employed for introducing variations in the protein sequence to attain the desired outcomes.

- **Selection and screening:**

After generating genetic diversity, the resulting mutants are typically expressed in bacterial or yeast hosts for protein production and then evaluated for functionality. Non-functional variants are removed using methods such as growth complementation, reporter-based systems, and display technologies (plasmid, phage, or ribosome display). Functional screening involves measuring the activity of each protein variant, often using high-throughput techniques. These include microtiter plate assays, digital imaging with spectroscopy, FACS (fluorescence-activated cell sorting), Förster resonance energy transfer (FRET), and cell surface display. Advanced analytical tools such as NMR, HPLC, mass spectrometry, and gas chromatography are also employed to assess activity and selectivity. The top-performing variants are selected and used as templates in subsequent rounds of mutagenesis. By systematically combining screening and selection strategies, protein variants with the desired activity can be efficiently identified and optimized.

- **Gene Amplification:**

In the final stage, the most functionally active mutant variant or a set of sequences is selected for PCR amplification. This selected sequence undergoes a repetitive cycle of mutagenesis, screening, selection, and gene amplification until a mutant with the specified properties outlined in the protein engineering goal is achieved[8].

Semi-rational Design: Recent progress in enzyme engineering has addressed the limitations of both knowledge-based design and random evolutionary techniques by integrating systematic enzyme modification with stochastic mutation approaches. To select promising target sites characterized by restricted amino acid variability, semi-rational strategies also known as smart or knowledge-based library designs utilize data on protein sequence, structure, and function, in addition to computational predictive techniques. Library sizes can be drastically reduced by focusing on specific amino acid positions, while libraries with higher functional content can be generated by considering evolutionary diversity, topological constraints, and mechanistic aspects that influence amino acid identity. The efficiency of biocatalysis tailoring can be greatly increased through semi-rational protein engineering. The creation of small, high-quality libraries can significantly reduce the need for high-throughput techniques in library analysis, in addition to usually requiring fewer rounds to find variants with the required phenotype. Furthermore, the lower number of variants opens up new opportunities for screening library members utilizing methods that aren't appropriate for a high-throughput system. Lastly, these strategic approaches move the discipline from a predictive to hypothesis-oriented approach by providing a conception foundation for predicting and interpreting experimental outcomes. To draw attention to the steadily increasing number of fruitful enzyme engineering investigations using computer-guided and semi-rational protein design.

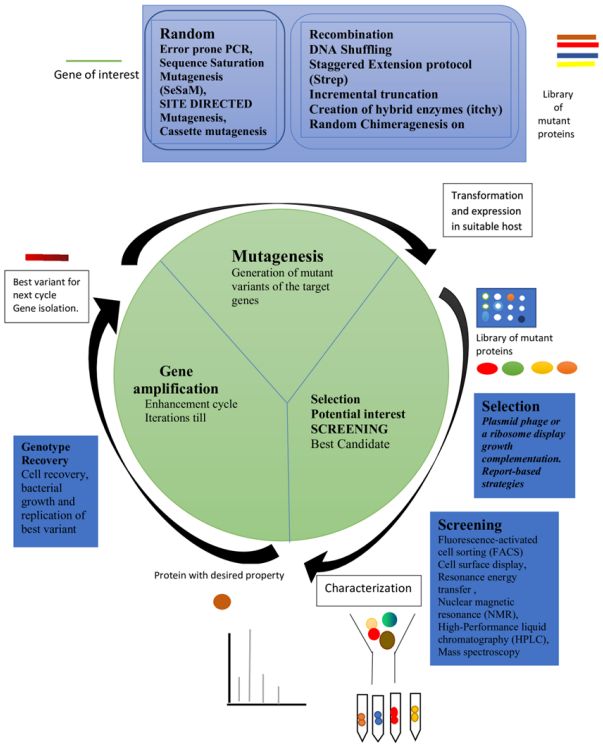


Fig. 5: Steps in the directed evolution

De novo design-De Novo Enzyme Design: A Pioneering Approach in Modern Enzyme Engineering. In enzyme engineering, de-novo design is a semi-rational method that uses computer-aided approaches to either generate new routes or reorganize existing ones. This approach mainly entails site-specific mutagenesis, achieved through the insertion and substitution of amino acids. Utilizing computational programs allows for the design of protein structures and the prediction of sequences that could improve the enzyme's biochemical properties[71]. The process relies on existing enzyme structures to generate new designs that can perform similar functions.

The initial step in de-novo design involves creating a new active site, using advanced computational tools to construct a site capable of facilitating a specific reaction's transition

state[72]. Following this, essential residues are incorporated to enhance stability, selectivity, and activity. This approach also allows for the redesign of non-covalent interactions, optimizing polarity and bonding, which enables novel substrates to bind to the enzyme's binding pockets at the desired site. The following sections provide a detailed examination of the core concepts and methodological steps involved in the de novo enzyme design process.

Table 3: Directed evolution in enzyme engineering for drug synthesis

Enzyme/Drug	Target enzyme	Mechanism of action	Directed evolution approach	Reference
Paclitaxel [Taxol] Synthesis	Taxadiene synthase	Catalyzes the first step in the biosynthesis of paclitaxel, a chemotherapy drug	Directed evolution to enhance enzyme activity and yield in microbial production of Taxol	67
Amylase variants	A-Amylase	Engineered amylases for enhanced thermostability and activity	Directed evolution of amylases to increase substrate affinity and improve thermal stability	68
Artemisinin synthesis	Artemisinic acid synthase	Catalyzes the formation of artemisinic acid, precursor to artemisinin, used in malaria treatment	Directed evolution to improve yield and optimize the enzyme for use in engineered yeast strains	69
Benzylisoquinoline Alkaloid Production	N-methyltransferase	Used in the synthesis of benzylisoquinoline	Directed evolution to enhance yield and specificity of alkaloid biosynthesis in microbial systems.	70

Role of Machine Learning and Artificial Intelligence (AI):

Biocatalysts, primarily enzymes, have become essential in modern synthesis due to their high selectivity, environmental compatibility, and cost-efficiency. Traditional methods for identifying and refining biocatalysts, such as random screening and directed evolution, have now been significantly enhanced by machine learning [ML], accelerating enzyme discovery and optimization. Machine learning involves computational techniques that recognize patterns in large datasets, including protein sequences, structures, and functional annotations. In biocatalysis, ML models predict enzymatic activity, stability, solubility, and substrate specificity, thus streamlining the identification of novel enzymes and reducing experimental failures. This predictive power has made ML indispensable in optimizing biocatalysts for various industrial and pharmaceutical applications[55].

Current ML models used in enzyme research include convolutional neural networks [CNNs], graph neural networks [GNNs], and transformer-based protein language models [PLMs] such as ESM and ProtTrans. These tools help in enzyme function prediction and activity forecasting, classifying unknown proteins and identifying promising variants for further validation. ML also reveals catalytic promiscuity, which uncovers secondary activities in enzymes, opening up new reaction pathways. Deep generative models like variational autoencoders [VAEs] and generative adversarial networks [GANs] extend ML's capabilities, enabling the generation of synthetic enzyme sequences. Combined with high-throughput data, these models support the design of tailored biocatalysts with desired properties for pharmaceutical, industrial, and environmental uses. Despite these advancements, challenges remain, including limited annotated datasets and difficulties in validating ML-predicted enzyme variants. However, the integration of ML with structural modeling and experimental biocatalysis holds significant potential for accelerating enzyme discovery and enhancing green synthetic processes[56].

PROCESS OPTIMIZATION AND IMPLEMENTATION

Enzyme Immobilization Technique and Nano biocatalysis:

Enzyme immobilization plays a key role in improving biocatalyst performance by enhancing stability, activity, and reusability. Common methods include carrier-based techniques where enzymes are attached to or enclosed within solid supports and carrier-free approaches like cross-linked enzymes [CLEs], along with techniques such as enzyme entrapment and membrane-based immobilization, which are visually represented in Fig. 6 of enzyme immobilization methods. While these methods simplify recovery and enable repeated use, weak enzyme-support interactions in aqueous systems can limit recyclability[74]. Enzyme entrapment techniques contain biocatalysts within matrices using diffusional or polymerization processes. This strategy shields enzymes from denaturation under challenging conditions and allows precise control over their placement within the matrix. Membrane-based immobilization is particularly beneficial for allosteric enzymes, although it exposes them to potential destabilizing agents like shear and hydrolytic activity.

All these methods enhance biocatalysis by stabilizing enzymes, facilitating their reuse, and improving their storage stability, thus broadening their applicability in various

industrial processes. Native enzymes, due to their water solubility, are expensive and difficult to recover from aqueous media, limiting their industrial applications. This limitation has been addressed through enzyme immobilization, transforming enzymes into heterogeneous biocatalysts. This approach offers benefits including high turnover, easy recovery, enhanced catalytic yield, stability, re-usability, cost-effectiveness and longer shelf-life. Immobilization also simplifies reaction procedures and aids in high-yield bio-product recovery with minimal environmental impact.

Numerous materials have been explored for enzyme immobilization, each with its advantages and disadvantages. Recent research focuses on using nanomaterials like nanofibers, nanoparticles, metal-organic frameworks [MOFs], hybrid nanoflowers, carbon-based nanotubes, and other nanostructures as support matrices for developing efficient biocatalysts. These nanomaterials provide functional groups such as hydroxyl, carboxylic, amine, thiol, and epoxy groups, facilitating the binding of enzymes to the matrices and enhancing characteristics like hyperactivity, stability, re-usability, and substrate affinity. Despite their advantages, nanomaterials also have limitations that must be carefully considered. Various strategies are being developed to overcome these limitations, including physical adsorption, entrapment in polymer networks, covalent attachment, and cross-linked enzyme aggregates [CLEAs]. MOFs, in particular, have gained attention due to their abundant functional groups that facilitate enzyme immobilization through physical adsorption or covalent attachment. Multipoint coupling is a robust strategy for enhancing enzyme-support linkages, addressing issues like conformational flexibility and enzyme denaturation.

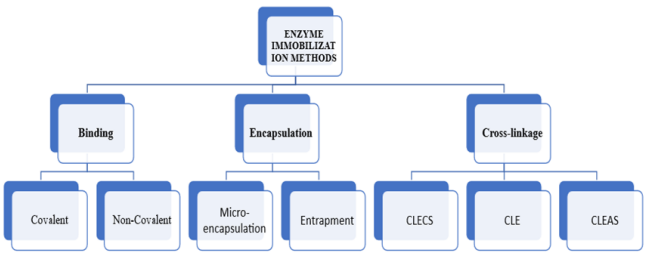


Fig. 6: Enzyme immobilization methods

Recent studies have demonstrated the effectiveness of MOFs in enzyme immobilization. For example, researchers developed nano-/microscale UiO-66-NH2 MOF materials that successfully immobilized soybean epoxide hydrolase, resulting in high enzyme loading, substrate affinity, and stability. Another study introduced metal-organic enzyme aggregates [MOEAs] as a promising carrier immobilization system with high transformation rates and thermostability, highlighting the potential of MOFs and related materials in industrial enzyme applications.

Nanobiocatalysis:

Enzymes and nanostructured materials are combined in nanobiocatalysis, emphasizing their close link. The increased surface area of nano-objects improves enzyme loading and bioconversion rates, among other benefits. Nanomaterials can be specifically tailored to match the characteristics of enzymes due to their precise physicochemical properties.
Enzymes connected to nanostructures such as nanotubes, nanoparticles, and mesoporous materials are used in nanofabrication processes. Mesoporous silicates are widely utilized due to its high surface area and resistance to harsh situations.

In the creation of nano-biocatalysts, "grafting from" and "grafting onto" are the two primary techniques. "Grafting from" refers to the process of derivatization once enzymes are affixed to nanostructures. "Grafting onto" refers to the use of self-assembly techniques to entrap enzymes in nanodevices. A new development is single enzyme nanoparticles [SEN], which have an encapsulating nanometric shell around the enzyme. This improves Enzyme stability and activity even in the most adverse circumstances. Biosensors with great selectivity, sensitivity, and tolerance to different environments have been developed using SEN technology.

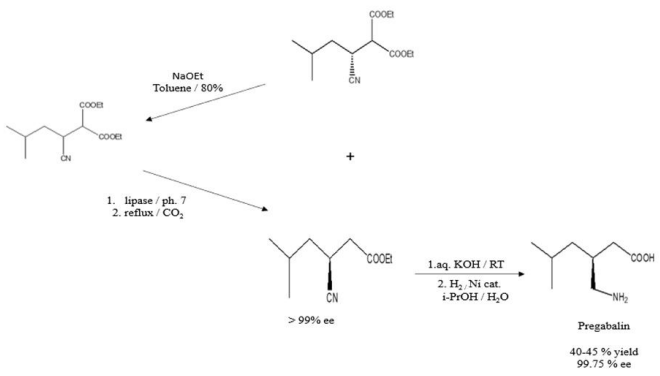
Enzyme Cascades and Chemoenzymatic Strategies:

Chemoenzymatic synthesis refers to the use of natural or engineered enzymes in combination with chemical catalysts to carry out organic transformations. In this approach, enzymes facilitate highly selective reactions, while chemocatalysts provide broader reactivity, making the combined process more efficient and versatile. The synergy between chemocatalysis and biocatalysis allows for improved reactivity, enhanced selectivity, and greater efficiency in synthetic pathways[36, 37].

This dual strategy is particularly valuable in pharmaceutical manufacturing, where it helps minimize, reaction steps, avoids the isolation of unstable intermediates, reduces costs, and supports environmentally sustainable production[38]. By integrating the strengths of both catalytic systems, chemoenzymatic methods address the limitations of each approach individually. As a result, this methodology offers a powerful tool for developing greener, scalable, and more economical synthetic processes, with growing potential across various industries[39-41].

The pivotal phase involves enzymatic kinetic resolution. of an ester using Thermomyces lanuginosus lipase [Lipolase]. The stereochemistry at C2 becomes inconsequential due to subsequent thermal decarboxylation. The unreacted substrate is racemized with sodium ethoxide in toluene at 80°C and recycled. Hydrolysis and hydrogenation lead to pregabalin with a 40-45% overall yield[42]. This

chemoenzymatic route significantly improves process efficiency, evident in a 7-fold decrease in the E factor [from 86 to 12] and a substantial reduction in organic solvent usage, attributed to a predominantly aqueous reaction medium. The key steps like enzymatic kinetic resolution, thermal decarboxylation, racemization, and recycling of unreacted substrate. This approach represents a more cost-effective and environmentally friendly method for producing pregabalin[43].



Scheme 1: The chemoenzymatic synthesis of pregabalin showcases a more cost-effective and environmentally friendly approach

Enzyme cascade:

An innovative development in biocatalysis, enzyme cascades enable the smooth coordination of several enzymatic events sequentially. These cascades have special benefits, like the capacity to replenish cofactors and guarantee that reactions are completed. Cascade modeling also makes it possible to optimize and fine-tune processes, which eventually results in increased yields of the desired goods.

Utilizing the innate capacity of enzymes to selectively catalyze reactions in aqueous settings, biocatalytic cascades effectively synthesize complex compounds by imitating the intricacy of natural biosynthesis routes. Recent developments in biocatalyst design have further increased this efficiency, making these cascades more extensively applicable and more easily available in a variety of industries.A biocatalytic cascade usually consists of two or more consecutive reactions, frequently without the requirement to segregate intermediates. The steps in the process are constructed, optimized, and designed in a cycle. Initially, the target compound's synthesis route is planned utilizing methods such as biocatalytic retrosynthesis, where enzyme selection is based on screenings, literature, or discovery efforts.

Depending on infrastructure and enzyme availability, the cascade can be conducted using entire cells, enzymes that have been purified or both. Before merging all steps, each is thoroughly verified separately. Lastly, the synthetic pathway is refined to improve efficiency and product quality through methods of optimization like process and protein engineering[75].

Biocatalysis vs. chemical catalysis based on efficiency, cost, and environmental impact:

Table 4: Comparison table of biocatalysis vs. chemical catalysis based on efficiency, cost, and environmental impact

Parameter	Biocatalysis [enzymes as catalysts]	Chemical catalysis
Reaction Efficiency	High specificity and selectivity; avoids side reactions	High activity but may produce unwanted byproducts
Reaction conditions	Mild reaction parameters like room temperature, aqueous solutions, and neutral pH	Requires high temperature, pressure, and harsh solvents
Catalyst regeneration	Often self-regenerating and reusable	May require additional purification and recycling steps
Substrate specificity	Highly selective for target molecules	Broad substrate range but lower selectivity
Cost	High initial cost but lower long-term cost due to reusability	Lower initial cost but higher operational cost due to harsh reagents.
Scalability	Improved with modern enzyme engineering and immobilization	Established protocols for large-scale synthesis.
Environmental impact	Biodegradable, renewable, non-toxic, low energy input	Generate hazardous waste, uses non-renewable metals, and solvents.
Sustainability	Aligns with green chemistry principles	Often energy-intensive and environmentally burdensome
Examples	Sitagliptin [merk], Atorvastatin intermediates, recent Ipatasertib synthesis using ketoreductase	Ibuprofen, paracetamol, and omeprazole still rely on chemical catalysis, although greener versions are in progress.

INDUSTRIAL IMPACT AND APPLICATIONS

Case Studies in Pharmaceutical Synthesis

- **Biocatalyst Application in Anti-Cancer Treatments:**

Biocatalysts, primarily enzymes, play a crucial role in accelerating chemical reactions, often enhancing reaction rates by up to 10^8 times. These enzymes are crucial in anticancer treatments, as they can selectively activate drugs

in the tumor microenvironment, minimizing harm to healthy cells. Additionally, nano-biocatalysts aid in tumor invasion, angiogenesis, and mutagenesis, while providing valuable data on microenvironmental markers' expression and activity. Biocatalysts are used in the production of various anticancer drugs, such as cinepazide, lysine dioxygenase, epothilone, and moclobemide. The application of biocatalysis has become essential in both academic and commercial sectors, particularly in green synthesis. This success is largely due to advancements in high-throughput laboratories and the discovery of new enzymes. By replacing traditional chemical catalysis, biocatalysis is increasingly seen as a sustainable and efficient alternative in cancer drug development, offering precision and selectivity in drug

activation. Specific drug examples and their biocatalyst combinations are detailed in Table. 5 [29].

- **Role of biocatalyst in tumor tissues:**

The diverse enzyme expression profiles in tumor tissues provide valuable targets for designing efficient enzyme-responsive nanodrug delivery systems. Designing a stimulus-responsive. The diverse patterns of enzyme expression found in tumor tissue help develop efficient enzyme-responsive nano-drug delivery devices. A strategy to concentrate a drug within tumors involves creating a stimulus-responsive system that releases the therapeutic agent specifically in the tumor microenvironment [TME]. The medication and its carrier can be connected using the enzyme substrate, which is present in the TME. Another option is to put the medication in a carrier that these TME enzymes can precisely break down. This approach offers advantages such as rapid drug release, enhanced penetration into tumor tissues, and improved therapeutic efficacy.

- **Role of Biocatalysts in Natural Product Synthesis:**

Biocatalysts have proven effective in the production of a wide range of compounds. Among the most important plant alkaloids [BIAs] are benzoyl isoquinoline alkaloids, which are produced from tyrosine or phenylalanine. The anticancer medication noscapine was first fully synthesized in yeast. To do this, 430 enzymes from a wide range of sources were expressed. Biocatalysts are also used in the manufacture of anti-cancer medications, vincristine and camptothecin. The enzyme strictosidine synthase [STR], which forms C-C bonds, produces strictosidine, the primary intermediate of MIAs. Strictosidine is formed through the conversion of the monoterpenoid secologanin and tryptamine, which is derived from tryptophan[30].

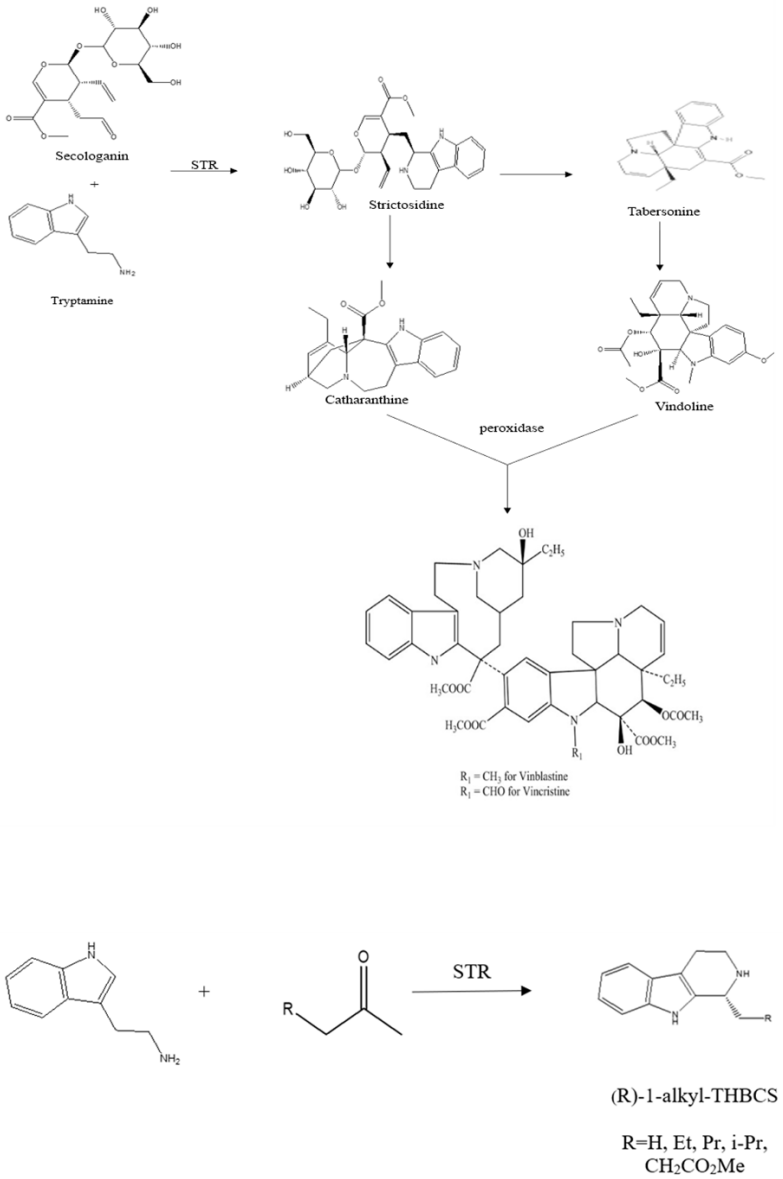
Synthesis of simvastatin:

Simvastatin, a widely used cholesterol-lowering drug marketed as Zocor by Merck, is produced through a semi-synthetic process. The synthesis begins with the chemical modification of lovastatin, a natural compound, which is cleaved to form monacolin J. This intermediate is then protonated to yield the acidic form of simvastatin. To install the dimethylbutyryl side chain, the hydroxyl group is first protected, followed by acylation. Finally, the protective group is removed, resulting in the active simvastatin product. The entire process requires six steps that are both technically and economically challenging. In contrast, the biocatalytic approach to synthesizing simvastatin is both efficient and straightforward, requiring only two steps. This novel approach uses the potent whole-cell acyltransferase LovD to precisely regioselectively acylate the C-8 hydroxyl group of monacolin J with α-dimethylbutyryl-S-N-acetylcysteamine [DMB-S-NAC], directly producing simvastatin. Interestingly, a hydrolase enzyme selectively cleaves the ester bond, but an acyltransferase stimulates ester synthesis in an aqueous environment, with both enzymes acting via very similar methods. Furthermore, simvastatin uniquely maintains all its chiral centers in the natural starting material, a feature not shared by other statins. Despite this, other statins can also be synthesized with a diverse array of biocatalysts, showcasing the versatility and potential of biocatalytic methods in pharmaceutical development[27].

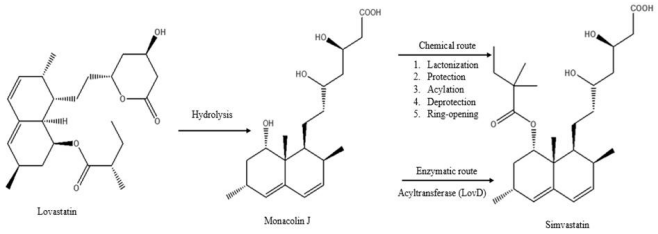
Table 5: Drugs and their combination used to develop a drug delivery system with biocatalysts for cancer therapy

Drugs	Drug-Delivery System	Biocatalysts used	Results [Targeted Site/ Nanomedicine Produces]	Ref no.
Doxorubicin	Mesoporous silica nanoparticle	MMP-13	Reduced side effects as a targeting moiety and end-capping agent.	31
Doxorubicin + verapamil	Transferrin-conjugated PEGylated liposome	Cytochrome P450 oxidase	To treat leukemia, the efficacy of liposomal loading was observed to be 95% and 70% of DOX and VER, respectively.	32
Gemcitabine + doxorubicin	HPMA-Gem- Dox	MMP-2	In prostate cancer, it was discovered that polymers in the form of liposomes might be used to transport numerous chemotherapeutic medicines to the tumor locations in vivo simultaneously.	33

Drugs	Drug-Delivery System	Biocatalysts used	Results [Targeted Site/ Nanomedicine Produces]	Ref no.
Unmethylated CpG-ONTs + doxorubicin	Aptamer-G4PAMAM dendrimer conjugates	Cytochrome P450 oxidase	A chemo-immunotherapy system for treating prostate cancer.	34
Vincristine	liposome	Strictosidine synthase [STR] to produce the intermediate Strictosidine, and peroxidase for the production of vincristine.	Marqibo [US FDA-approved nanomedicines]	35



Scheme 2: Synthesis of monoterpenoid indole alkaloids. The strictosidine synthase [STR] enzyme is used in portion (A) to transform monoterpenoid secologanin and tryptophan-derived tryptamine into strictosidine, an important MIA intermediate. After that, strictosidine undergoes several changes that result in the creation of multiple MIA subclasses. It is shown in part (B) that by replacing secologanin with some basic aliphatic aldehydes, a small number of STRs produce (R)-1-alkyl THBCs in medium to high optical purity



Scheme 3: Comparing chemical and biocatalytic synthesis of simvastatin

Table 6: Biocatalytic Alcoholysis of Steroid Diesters Using *Candida cylindracea* Lipase [CCL]

Parameter	Description
Biocatalyst	*Candida cylindracea* lipase [CCL]
Selectivity	Exhibits high site-selectivity by preferentially hydrolyzing the ester at the 17α-position
Operating Conditions	Mild temperatures [20°C–32°C], carried out in toluene over approximately 53 hours
Substrate	17α,21-dibutanoate derivative of 9,11-dehydrocortexolone
Alcohol Used	Methanol is utilized as the nucleophile in the transesterification reaction
Reaction Type	Lipase-catalyzed selective ester cleavage [alcoholysis]
Main Product	17α-monoester derivative
Yield and Efficiency	**79% yield of the 17α-selective monoester.**
Key Benefits	• Maintains molecular structure under gentle conditions • Eliminates the need for protecting groups • Streamlined purification process • Environmentally friendly and suitable for scale-up • Reduces hazardous reagents and aligns with sustainable and regulatory manufacturing goals

Enzymatic Method for Producing 17α-Monoesters of Cortexolone and/or its 9,11-Dehydroderivatives

Enzymatic Synthesis of 17α-Monoesters of Cortexolone Derivatives: A Sustainable Approach for Pharmaceutical Manufacturing: Current developments in biocatalysis have resulted in considerable improvements in the production of complex medicinal molecules. One noteworthy development, as documented in U.S. Patent US11,938,141 B2, presents an innovative enzymatic process for the synthesis of 17α-monoesters of cortexolone and its 9,11-dehydro derivatives, which hold substantial promise for applications in anti-inflammatory and anti-androgenic therapies.

Background: Corticosteroid Derivatives in Pharmaceutical Applications

Cortisol derivatives, particularly 17α-monoesters, are crucial in the pharmaceutical industry for treating conditions like acne and psoriasis due to their potent anti-inflammatory and anti-androgenic effects. Traditional chemical synthesis methods often involve multiple steps, harsh reagents, and high temperatures, resulting in low yields, undesired by-products, and poor selectivity.

A patented enzymatic method offers a more efficient and sustainable approach to producing 17α-monoesters from 17α,21-diesters of cortexolone or its 9,11-dehydro derivatives. Using Candida cylindracea lipase [CCL] as the biocatalyst, this process provides high specificity and regioselectivity in ester hydrolysis reactions, improving the overall efficiency of production[28].

Successful Biocatalysis in the Pharmaceutical Industry: Engineered Cytochrome P450s:

Cytochrome P450 [CYP] enzymes represent one of the most notable examples of successful biocatalysis in the pharmaceutical industry, particularly in the fields of drug metabolism and synthesis. These enzymes are integral to many pharmaceutical applications, and their engineering has catalyzed significant advancements.

- **Role of Cytochrome P450 Enzymes**

Cytochrome P450 enzymes are a large superfamily that catalyze the oxidation of organic molecules. In drug metabolism, their main function is to convert lipophilic substances into hydrophilic metabolites, facilitating their excretion from the body. This process is crucial for drug clearance and detoxification. Beyond metabolism, P450 enzymes are versatile biocatalysts capable of performing complex reactions like hydroxylation, epoxidation, and demethylation, reactions that are often difficult to achieve using traditional chemical methods. This makes P450 enzymes valuable tools in pharmaceutical research and development[51].

- **Engineering Cytochrome P450 for Enhanced Specificity and Efficiency:**

Research on cytochrome P450 enzyme engineering focuses on improving their specificity, efficiency, and stability for pharmaceutical use. Techniques like site-directed mutagenesis allow modifications of amino acid residues in

the enzyme's active site, tailoring its catalytic properties[52]. This enables P450s to selectively hydroxylate specific positions on drug molecules, optimizing their pharmacokinetics and pharmacodynamics. Engineered P450s can perform regio- and stereoselective oxidations, crucial for synthesizing complex pharmaceuticals, processes that traditional methods often cannot achieve.

- **Impact on Drug Development:**

Engineered cytochrome P450 enzymes have transformed drug development by simplifying the synthesis of complex pharmaceutical compounds. Biocatalysis offers key advantages over traditional chemical methods, such as higher yields, enhanced product purity, and reduced environmental impact. The mild conditions associated with enzymatic catalysis—such as ambient temperature and pressure—make the process more sustainable by minimizing energy use and hazardous waste. Additionally, P450 enzymes enable challenging synthetic transformations, opening new avenues for creating novel drug scaffolds and expanding drug discovery possibilities[53].

- **Case Studies and Industrial Applications:**

Case studies demonstrate the successful use of engineered cytochrome P450 enzymes in pharmaceutical manufacturing, particularly in synthesizing intermediates for anti-cancer drugs. These enzymes excel at regioselective hydroxylation and are crucial for producing chiral compounds, ensuring the efficacy and safety of therapeutics. Their stereoselectivity is especially valuable in creating enantiomerically pure compounds, as the chirality of drugs influences their biological activity. By adopting biocatalysis, pharmaceutical manufacturers improve product quality and enhance therapeutic potential[54].

Alignment with the 12 Principles of Green Chemistry and White Biotechnology:

- **Role of Biocatalysis in Green Chemistry:**

Understanding Green Chemistry and Its Principles: Green chemistry focuses on designing chemical products and processes that minimize the use and generation of hazardous substances. Its primary aim is sustainability in chemical manufacturing and reducing environmental impact[11]. The twelve core principles that guide this field are summarized in Fig. 7 of Principles of Green Chemistry.

Key Principle of White Biotechnology:

White biotechnology, or industrial biotechnology, harnesses biological systems for environmentally responsible industrial processes[12]. Its core principles are included in the Fig. 8.

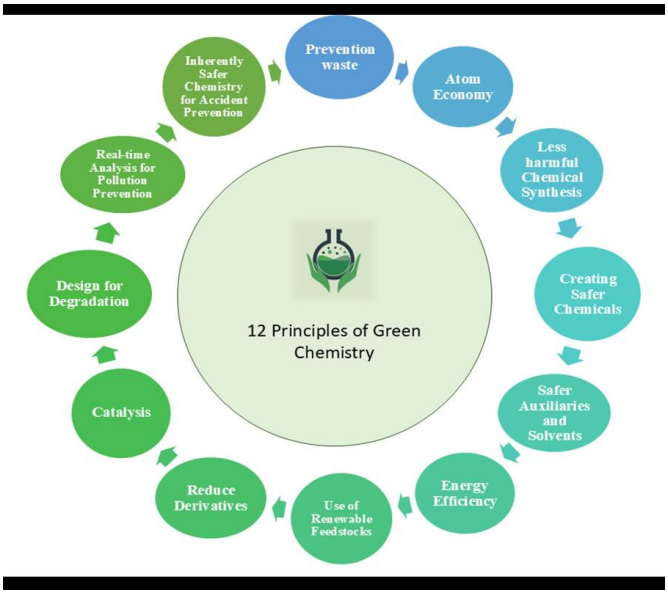


Fig. 7: 12 Principles of Green Chemistry

Fig. 8: Principles of white biotechnology

FUTURE TRENDS AND OUTLOOK

Regulatory Acceptance and FDA Perspective:

Biocatalysts are gaining recognition from regulatory agencies like the FDA due to their efficiency, selectivity, and environmentally friendly nature in pharmaceutical manufacturing. Their use supports sustainable and innovative production methods. Progress in molecular biology and enzyme engineering has broadened their role in drug development, as they enable complex chemical reactions under mild, eco-friendly conditions. The FDA increasingly supports biocatalysis for its ability to enhance efficiency, reduce hazardous waste, and improve drug

quality and safety. Furthermore, biocatalysis can shorten development timelines and lower costs, helping bring new treatments to market more quickly[15].Despite growing acceptance, challenges remain in standardizing processes and ensuring enzyme performance across scales. The FDA is developing guidelines to integrate biocatalysis into pharmaceutical manufacturing effectively[16]. A list of biocatalytic drugs that have received recent approval from the FDA is presented in Table. 7.

Table 7: Recent FDA-Approved Biocatalytic Drugs

Drug Name	Therapeutic use	Biocatalytic role	Manufacturer	Reference
Rezafungin [Rezzayo]	Treatment of candidemia and invasive candidiasis	Inhibits fungal β-glucan synthase enzyme [echinocandin class antifungal]	Cidara therapeutics	17
Molnupiravir [lagevrio]	Treatment of covid-19	Biocatalytic cascade synthesis of nucleoside analog [via engineered enzymes]	Merk & cp., Inc	18
Pombiliti [cipaglucosidase alfa-atga]	Late-onset Pompe disease therapy	Recombinant human acid αglucosidase enzyme replacement	Amicus	19
Ryzneuta [efbemalenograstim alfa-vuxw]	Chemotherapy-induced neutropenia prevention	Recombinant G-CSF fusion protein stimulates neutrophil production.	Evive Biotech	20
	Monoclonal antibodies			
Crovalimab [PiaSky]	Paroxysmal nocturnal hemoglobinuria	Complement C5 inhibitor; blocks terminal complement cascade to prevent hemolysis.	Genentech [Roche]	21
Zolbetuximab [Vyloy]	Initial therapy for adults with locally advanced unresectable or metastatic HER2-negative, gastric or gastroesophageal junction adenocarcinoma expressing CLDN 18.2, used in combination with chemotherapy	A cytolytic antibody specifically targeting tumor cells that express Claudin 18.2 [CLDN18.2]	Astellas pharma Us, Inc.	22
Rozanolixizumab [Rystiggo]	Generalized myasthenia gravis	Inhibits FcRn-mediated IgG recycling [modulation of the immune pathway]	UCB, Inc.	23
Cosibelimab [unloxcyt]	Metastatic or locally advanced cutaneous squamous cell carcinoma in adults not candidates for curative surgery or radiation[24].	Programmed death-ligand 1 [PD-L1] blocking antibody restores anti-tumor immune response.	Checkpoint Therapeutics, Inc.	25
Durvalumab [Imfinzi]	Approved for treating unresectable hepatocellular carcinoma and biliary tract cancer	Blocks PD-L1, enhancing T-cell anti-tumor activity.	AstraZeneca.	26

CONCLUSION

Biocatalysis offers a transformative alternative to conventional chemical synthesis by enabling efficient, selective, and environmentally responsible processes. With the integration of enzyme engineering, computational modeling, and green chemistry principles, it is now possible to design customized catalysts for complex chemical transformations. These advancements have significantly improved yields, reduced hazardous waste, and enabled synthesis under mild, energy-efficient conditions. The successful industrial application of biocatalysis, particularly in the pharmaceutical sector, underscores its scalability and reliability. Furthermore, recent innovations such as machine learning-driven enzyme design, nano-biocatalysts, and chemoenzymatic synthesis have opened new avenues for process intensification and sustainable manufacturing. As global industries shift toward greener technologies,

biocatalysis stands as a critical enabler of eco-friendly and cost-effective production. Looking ahead, continued interdisciplinary research and investment will be crucial to further expand its scope, address current limitations, and enhance its impact across industrial chemistry.

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