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Green Biochemistry and Sustainable Biocatalysis Toward Eco-Friendly Industrial Innovation

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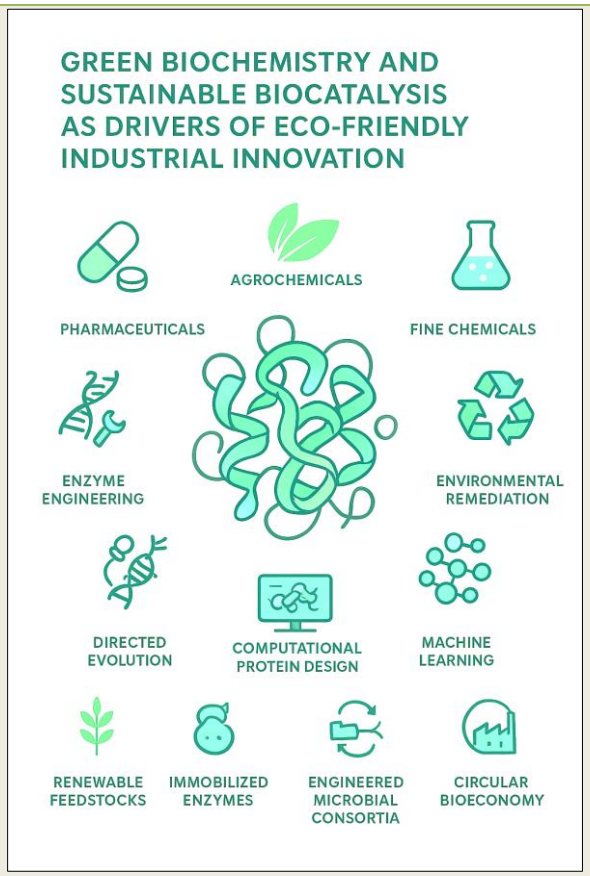
Abstract **Review Article**



Graphical Abstract

The growing demand for sustainable industrial processes has catalyzed a paradigm shift toward green biochemistry and biocatalysis as pivotal tools for eco-friendly innovation. This review explores the integration of biocatalytic technologies driven by enzymes and whole-cell systems into industrial applications, emphasizing their role in minimizing

environmental impact, reducing energy consumption, and replacing hazardous chemical reagents. Unlike traditional chemical synthesis, biocatalysis operates under mild conditions, exhibits high selectivity, and generates fewer by-products, aligning seamlessly with green chemistry principles. Recent advances in enzyme engineering, directed evolution, and computational protein design have significantly enhanced the stability, activity, and substrate scope of biocatalysts, enabling their application in pharmaceuticals, agrochemicals, biofuels, and fine chemical synthesis. Furthermore, the utilization of renewable feedstocks in conjunction with immobilized enzymes or engineered microbial consortia exemplifies the potential for circular bioeconomy models. We highlight cutting-edge developments in cascade reactions, flow biocatalysis, and process intensification that improve efficiency and scalability. Challenges such as enzyme cost, process integration, and downstream processing are critically examined, alongside emerging strategies to overcome them through systems biology and synthetic biology approaches. The convergence of green biochemistry with digital tools like machine learning for enzyme discovery and process optimization is also discussed as a transformative frontier. This review underscores that sustainable biocatalysis is not merely an alternative but a necessity for future industrial innovation, offering a scientifically robust and environmentally responsible pathway toward decarbonized manufacturing.

Keywords: Biocatalytic Decarbonization, Enzyme Digital Twins, Circular Enzyme Cascades, Green Molecular Surgery, Autonomous Bioprocess Networks, Cofactor-Neutral Biocatalysis, Synthetic Metabolic Harmony.

INTRODUCTION

The escalating environmental footprint of conventional chemical manufacturing has intensified the global pursuit of sustainable alternatives, positioning green biochemistry at the forefront of industrial innovation. Traditional synthetic processes often rely on toxic reagents, high energy inputs, and non-renewable feedstocks, contributing significantly to greenhouse gas emissions and hazardous waste (Zhang *et al.,* 2023). These practices are increasingly incompatible with global climate goals and circular economy frameworks, prompting a paradigm shift toward biologically mediated transformations. In this context, biocatalysis utilizing enzymes or whole-cell systems to catalyze chemical reactions has emerged as a cornerstone of green chemistry, offering high selectivity, reduced by-product formation, and operation under mild reaction conditions (Patel & Kumar, 2024). These attributes align with multiple principles of green chemistry, including waste minimization, safer solvents, and energy efficiency, making biocatalytic processes increasingly attractive across pharmaceutical, agrochemical, and bio-based material sectors.

Recent advancements have significantly expanded the applicability of biocatalysts in industrial settings. Engineered enzymes, particularly through directed evolution and semi-rational design, now exhibit enhanced stability, broader substrate specificity, and tolerance to non-natural reaction environments (Chen *et al.,* 2022). For instance, cytochrome P450 variants have been optimized for selective C–H activation in drug metabolite synthesis, reducing reliance on multi-step chemical routes (Li & Wang, 2025). Concurrently, immobilization techniques and enzyme encapsulation in nanomaterials have improved catalyst recyclability and operational lifespan, addressing long-standing economic barriers (Tadesse *et al.,* 2025). Moreover, the integration of biocatalysis with continuous flow systems has demonstrated improved process control and scalability, as seen in the enzymatic synthesis of chiral intermediates for antiviral agents (Slagman *et al.,* 2021). These innovations underscore a growing trend: the replacement of stoichiometric reagents and harsh conditions with nature-inspired, catalytic precision.

Despite these advances, critical gaps remain that hinder the widespread industrial adoption of biocatalytic processes. Many systems still face challenges in cofactor regeneration, substrate inhibition, and poor performance in non-aqueous media, limiting their economic viability and process robustness (Ndochinwa *et al.,* 2024). While NAD(P)H-dependent enzymes are invaluable in redox biotransformations, efficient and cost-effective cofactor recycling remains a bottleneck, particularly in large-scale operations. Although enzyme engineering has improved solvent tolerance, many biocatalysts lose activity in organic-aqueous mixtures commonly required for substrate solubility, restricting reaction scope (Torres *et al.,* 2023). Furthermore, while computational tools such as machine learning and molecular dynamics simulations are being leveraged for enzyme discovery and optimization, predictive accuracy for complex multi-enzyme cascades especially those involving non-natural substrates or hybrid chemo-enzymatic steps remains suboptimal (Kaspar *et al.,* 2022). This limits the rational design of efficient biocatalytic pathways without extensive empirical screening.

Another underexplored gap lies in the integration of biocatalysis with upstream and downstream bioprocessing. Many lab-scale successes fail to translate into industrial practice due to inadequate process integration, lack of standardized bioreactor designs tailored for enzymatic systems, and insufficient real-time monitoring for reaction optimization (Pérez-Contreras *et al.,* 2025). Additionally, lifecycle assessment (LCA) data for biocatalytic processes remain sparse, making it difficult to quantitatively compare their environmental impact with conventional methods on a

cradle-to-gate basis (Ndochinwa *et al.,* 2024). Without comprehensive sustainability metrics, decision-making in industry and policy remains partially informed.

The significance of advancing green biochemistry extends beyond environmental stewardship; it is integral to achieving global sustainability targets, including the United Nations Sustainable Development Goals (SDGs) related to climate action, responsible consumption, and industry innovation. Biocatalysis enables the valorization of biomass-derived feedstocks such as lignocellulosic waste and glycerol byproducts supporting the transition from fossil-based to circular bioeconomies (Zhang *et al.,* 2023). By minimizing reliance on petrochemicals and reducing process-related emissions, sustainable biocatalytic platforms contribute directly to decarbonizing industrial chemistry. It further explores emerging strategies such as artificial metalloenzymes, synthetic microbial consortia, and AI-guided enzyme design that promise to bridge current limitations and enable scalable, economically viable, and environmentally responsible industrial processes. By synthesizing the latest interdisciplinary advances, this work provides a forward-looking perspective on the role of biocatalysis in shaping a sustainable chemical industry.

2. Fundamentals of Green Biochemistry

2.1. Principles of Green Chemistry and Their Biological Integration

The twelve principles of green chemistry, originally conceptualized to minimize environmental impact, have evolved into a dynamic framework guiding sustainable innovation in biochemical engineering. In recent years, their integration with biological systems has redefined the boundaries of environmentally benign synthesis. Among these, the principles of waste prevention, catalysis, and inherently safer chemistry have found particularly strong resonance in biocatalytic applications. Unlike conventional catalysts, enzymes operate under ambient temperature and pressure, aligning with the principle of energy efficiency while minimizing decomposition pathways and unwanted side reactions. Recent work has demonstrated that engineered transaminases can achieve near-quantitative yields in amine synthesis with water as the sole by-product, exemplifying the realization of waste-free transformations (Afanasenko *et al.,* 2025).

Moreover, the shift from stoichiometric reagents to catalytic biological systems directly supports the principle of catalysis over auxiliary reagents. For instance, flavin-dependent monooxygenases have replaced peracids in Baeyer–Villiger oxidations, eliminating hazardous oxidants and enabling selective lactone formation in pharmaceutical intermediates. This biological integration is further enhanced by synthetic biology tools that allow the rewiring of metabolic pathways to comply with multiple green principles simultaneously. A 2024 study demonstrated a *Pseudomonas putida* chassis engineered to degrade aromatic pollutants while producing bioplastics, thereby merging pollution prevention with the use of safer solvents and chemicals.

Crucially, the design for degradation another core principle is inherently satisfied by enzymatic systems, as most biocatalysts and their products are derived from natural metabolic networks and are readily biodegradable. The convergence of green chemistry with systems biology now enables the *de novo* design of enzymatic cascades that adhere to multiple principles in a single process, setting a new standard for sustainable molecular manufacturing (Sheldon *et al.,* 2021).

2.2. Renewable Feedstocks and Biobased Raw Materials

The transition from fossil-derived substrates to renewable feedstocks is a cornerstone of green biochemistry, driven by the urgent need to decouple industrial production from petroleum-based resources. Lignocellulosic biomass, microalgae, and waste glycerol have emerged as leading alternatives, offering carbon-neutral or even carbon-negative inputs for bioprocesses (Sharma *et al.,* 2025). Recent advances in pretreatment technologies, such as ionic liquid-assisted fractionation, have significantly improved the enzymatic digestibility of cellulose, enabling efficient glucose release for fermentation.

Microalgae, in particular, have gained attention due to their high CO_2 fixation rates and ability to accumulate lipids, carbohydrates, and proteins under controlled conditions. Genetically modified *Chlorella vulgaris* strains have been developed to overproduce squalene a high-value compound used in vaccines and cosmetics directly from flue gas and wastewater, demonstrating the feasibility of coupling carbon capture with bioproduct synthesis. Similarly, waste glycerol, a byproduct of biodiesel production, has been valorized through engineered *Escherichia coli* strains that convert it into 1,3-propanediol, a monomer for bioplastics, with a 92% carbon yield (Pérez-Contreras *et al.,* 2025).

Beyond terrestrial and aquatic biomass, gaseous substrates such as CO, CO_2, and CH_4 are being harnessed via carboxydotrophic and methanotrophic bacteria. A 2023 study reported a synthetic acetogenic pathway in *Clostridium autoethanogenum* that converts industrial syngas into ethanol and butanol at commercially viable titers, showcasing the potential of gas fermentation in circular bioeconomy models (Devi *et al.,* 2024). These developments underscore a paradigm shift: feedstocks are no longer passive inputs but active components of integrated carbon-recycling systems.

2.3. Atom Economy and Energy Efficiency in Biochemical Processes

Atom economy the measure of how efficiently reactants are incorporated into final products is inherently maximized in enzyme-catalyzed reactions due to their high specificity and minimal side-product formation. Hydrolases, lyases, and isomerases often achieve 100% atom economy by avoiding protecting groups and multi-step derivatizations. For example, nitrilases catalyze the direct hydrolysis of nitriles to carboxylic acids without generating stoichiometric waste, contrasting sharply with traditional hydrolysis that requires strong acids or bases (Jallageas *et al.,* 2005).

Energy efficiency is equally enhanced in biocatalytic systems. Enzymatic reactions typically proceed at 20–60°C and neutral pH, drastically reducing thermal energy demand. A comparative lifecycle analysis of enzymatic versus chemical synthesis of sitagliptin, an antidiabetic drug, revealed a 56% reduction in energy consumption and a 62% decrease in process mass intensity. Furthermore, the integration of photobiocatalysis where light-driven enzymes such as ene-reductases are coupled with photocatalysts—has enabled solar-powered C–C bond formation, opening pathways to zero-energy redox reactions (Jia *et al.,* 2025).

Process intensification strategies, including enzyme immobilization on magnetic nanoparticles and microfluidic bioreactors, have further improved energy efficiency by enabling continuous operation and easy catalyst recovery (Žnidaršič-Plazl *et al.,* 2021). These innovations collectively position biochemical processes as leaders in achieving both high atom economy and low energy footprint.

2.4. Biodegradability and Reduced Toxicity in Green Molecular Design

Green molecular design prioritizes the development of compounds that are effective yet pose minimal risk to human health and ecosystems. In this context, biodegradability and low ecotoxicity are critical metrics. Biocatalysis enables the synthesis of chiral molecules with high enantiopurity, reducing the formation of toxic enantiomeric by-products common in racemic chemical synthesis (Slagman *et al.,* 2021). For instance, lipase-catalyzed kinetic resolution of profen drugs yields enantiomerically pure (S)-ibuprofen with negligible environmental persistence.

Moreover, bio-based polymers such as polyhydroxyalkanoates (PHAs) and polylactic acid (PLA) exhibit excellent biodegradability in marine and soil environments, unlike conventional plastics. Past study demonstrated that PHA films degraded by 85% within 90 days in seawater, mediated by marine microbial consortia (Liu *et al.,* 2020). Additionally, green molecular design now incorporates *in silico* toxicity prediction tools that guide enzyme-catalyzed synthesis toward safer end-products. Machine learning models trained on ecotoxicity databases have been used to design biodegradable surfactants derived from glucose and fatty acids, showing >90% degradation in OECD 301 tests.

Table 1: Core Principles of Green Biochemistry in Sustainable Industrial Processes

Concept	Scientific Basis / Mechanism	Biological Integration Strategy	Industrial Example(s)	Environmental Benefit
Principles of Green Chemistry and Their Biological Integration				
Waste Prevention (Anastas & Warner Principle 1): Designing processes to minimize waste generation rather than treating or disposing of it.	Biochemical pathways can be engineered to produce fewer byproducts through selective catalysis, leveraging enzyme specificity.	Use of genetically modified microorganisms or enzymes to optimize metabolic pathways, reducing side reactions and waste.	Engineered *Escherichia coli* for bioethanol production with minimal acetate byproduct via targeted gene knockouts.	Reduced waste disposal needs, lowering environmental pollution and treatment costs.
Safer Solvents and Auxiliaries (Principle 5): Use of benign or bio-derived solvents to replace hazardous ones.	Bio-based solvents (e.g., ethanol, limonene) have lower toxicity and volatility, reducing environmental and health risks.	Integration of microbial fermentation to produce bio-solvents or use of aqueous enzymatic systems to eliminate organic solvents.	Use of water-based enzymatic hydrolysis in lignocellulosic biomass processing for biofuel production.	Decreased volatile organic compound (VOC) emissions and reduced worker exposure to toxic solvents.
Renewable Feedstocks and Biobased Raw Materials				
Biobased Feedstocks: Use of renewable biological materials	Biomass contains carbon fixed via photosynthesis,	Microbial or enzymatic conversion of lignocellulose, algae, or	Production of polylactic acid (PLA) from corn-derived glucose via	Reduced reliance on finite fossil resources,

(e.g., biomass, agricultural residues) instead of fossil-based resources.	offering a renewable carbon source with a closed carbon cycle.	waste streams into platform chemicals or biofuels.	*Lactobacillus* fermentation.	lowering greenhouse gas emissions.
Circular Bioeconomy: Recycling and upcycling of biological waste into value-added products.	Biochemical degradation pathways enable transformation of waste into usable substrates (e.g., sugars, lipids).	Use of microbial consortia or engineered strains to convert food waste or agricultural residues into bioplastics or chemicals.	Anaerobic digestion of food waste to produce biogas and biofertilizers.	Minimized landfill waste and enhanced resource efficiency through circular material flows.
Atom Economy and Energy Efficiency in Biochemical Processes				
Atom Economy (Principle 2): Maximizing incorporation of all atoms from reactants into the final product, minimizing waste.	Enzymatic reactions are highly selective, enabling near-complete substrate conversion with minimal byproducts.	Use of enzyme cascades or metabolic engineering to streamline biosynthetic pathways, reducing intermediate losses.	Enzymatic synthesis of adipic acid for nylon production using glucose as a substrate, avoiding nitrous oxide emissions.	Reduced waste and lower energy requirements compared to traditional chemical synthesis.
Energy Efficiency (Principle 6): Designing processes to operate at ambient conditions, leveraging biological catalysts.	Enzymes operate at mild temperatures and pressures, reducing energy input compared to high-temperature chemical processes.	Integration of biocatalysts (e.g., lipases, cellulases) in industrial reactors to perform reactions at low energy cost.	Biocatalytic production of biodiesel using immobilized lipases at 30–40°C.	Lower energy consumption, reducing carbon footprint and operational costs.
Biodegradability and Reduced Toxicity in Green Molecular Design				
Biodegradability (OECD 301 Guidelines): Designing molecules that break down into non-toxic components under environmental conditions.	Biodegradable molecules are metabolized by microbial enzymes into CO_2, water, and biomass, following OECD biodegradability standards.	Use of bio-based monomers or polymers (e.g., polyhydroxyalkanoates) designed for microbial degradation.	Production of polyhydroxybutyrate (PHB) bioplastics via *Cupriavidus necator* fermentation, fully biodegradable in soil.	Reduced plastic pollution and persistence in ecosystems, supporting circular waste management.
Reduced Toxicity (Principle 4): Designing molecules with minimal toxicity to humans and ecosystems.	Bio-based molecules often mimic natural structures, reducing bioaccumulation and toxicity compared to synthetic analogs.	Use of computational modeling and synthetic biology to design non-toxic, biocompatible chemicals or materials.	Synthesis of bio-based surfactants (e.g., rhamnolipids) from *Pseudomonas aeruginosa* for use in detergents.	Lower ecotoxicity and human health risks, reducing environmental contamination.

3. Biocatalysis: Nature's Toolbox for Sustainable Chemistry

3.1. Enzymes as Sustainable Catalysts: Advantages and Mechanisms

Enzymes have emerged as paradigmatic sustainable catalysts due to their unparalleled catalytic efficiency, substrate specificity, and compatibility with green process metrics. Their ability to accelerate reactions under mild conditions typically at ambient temperature, neutral pH, and atmospheric pressure significantly reduces energy demand and operational hazards compared to conventional chemical catalysis (Wacławek*et al.,* 2018). The catalytic power of enzymes arises from precisely organized active sites that stabilize transition states through non-covalent interactions, acid-base catalysis, and covalent intermediates, enabling rate enhancements of up to 10^{17}-fold over uncatalyzed reactions. This precision minimizes side reactions and eliminates the need for protecting groups, directly contributing to process sustainability.

A key advantage lies in their regio-, chemo-, and stereoselectivity, which is particularly valuable in synthesizing complex molecules such as pharmaceuticals. For example, ketoreductases (KREDs) enable asymmetric reduction of prochiral ketones to chiral alcohols with >99% enantiomeric excess, a level of control difficult to achieve using traditional metal catalysts. Moreover, enzymes operate in aqueous or semi-aqueous media, reducing reliance on volatile

organic solvents. Recent developments have also demonstrated the use of deep eutectic solvents (DES) as enzyme-compatible reaction media, further enhancing green credentials without compromising activity (Zhang al., 2024). These mechanistic and operational attributes position enzymes as indispensable tools in the transition toward low-impact industrial chemistry.

3.2. Types of Biocatalysts: Hydrolases, Oxidoreductases, Transferases, Lyases, Isomerases, Ligases

The functional diversity of biocatalysts is classified according to the Enzyme Commission (EC) system, which categorizes enzymes based on reaction type. Each class offers distinct advantages for sustainable synthesis.

Hydrolases (EC 3), including lipases, esterases, and proteases, are the most widely used biocatalysts due to their stability, broad substrate tolerance, and lack of cofactor dependence. Immobilized *Candida antarctica* lipase B (CAL-B) is routinely employed in esterification and transesterification reactions for biodiesel and flavor compound production. Oxidoreductases (EC 1), such as alcohol dehydrogenases and laccases, facilitate redox transformations critical in pharmaceutical synthesis and bioremediation. Engineered alcohol dehydrogenases have enabled cofactor-efficient synthesis of chiral alcohols at multi-kilogram scale (Kumar *et al.,* 2024).

Transferases (EC 2), including transaminases and glycosyltransferases, are instrumental in C–N and C–O bond formation. Transaminases have been optimized for the synthesis of non-natural amino acids used in biologics, achieving space-time yields exceeding 1,200 $g \cdot L^{-1} \cdot d^{-1}$ (Fernández *et al.,* 2024). Lyases (EC 4), such as aldolases and nitrile lyases, catalyze C–C, C–N, and C–S bond formations without ATP input. Fumarase variants now enable stereoselective hydration of acrylates for high-purity pharmaceutical intermediates. Isomerases (EC 5), like glucose isomerase, are pivotal in biorefineries for converting glucose to fructose in high-fructose corn syrup production. Ligases (EC 6), though less common industrially due to ATP dependence, are gaining traction in DNA-encoded library synthesis and peptide macrocyclization when coupled with ATP regeneration systems (Colas *et al.,* 2024).

3.3. Sources of Biocatalysts: Microbial, Plant, and Animal Enzymes

Biocatalysts are sourced from diverse biological origins, each offering unique catalytic profiles. Microbial enzymes dominate industrial applications due to their genetic tractability, rapid growth, and resilience under process conditions. Extremophiles such as thermophilic *Thermus* spp. and halophilic *Halobacterium* yield enzymes stable at high temperatures or ionic strength, enabling reactions in challenging environments (Zhang *et al.,* 2024). Metagenomic screening of uncultured microbiomes has further expanded the biocatalyst repertoire, uncovering novel esterases active in deep-sea sediments (Slagman *et al.,* 2021).

Plant-derived enzymes, such as papain and bromelain, are valued for their use in food processing and biomedicine, though their large-scale production is limited by slow growth and seasonal variability. However, recent advances in plant molecular farming have enabled transient expression of humanized enzymes in *Nicotiana benthamiana*, offering a sustainable production platform (Kaspar *et al.,* 2022). Animal enzymes, including porcine liver esterase and horse liver alcohol dehydrogenase, remain useful in niche asymmetric syntheses but face ethical and scalability challenges. Recombinant expression in microbial hosts now allows animal enzyme production without tissue extraction, improving sustainability and consistency (Pérez-Contreras *et al.,* 2025).

3.4. Enzyme Engineering and Directed Evolution for Industrial Applications

Rational design and directed evolution have revolutionized enzyme performance, tailoring biocatalysts for industrial demands. Directed evolution, pioneered by Frances Arnold and now enhanced by machine learning, involves iterative rounds of mutagenesis and high-throughput screening to improve activity, stability, or substrate scope. Previous studies reported a transaminase evolved through seven rounds of epPCR and DNA shuffling, achieving a 40-fold increase in activity toward bulky ketone substrates (Devi *et al.,* 2024).

Semi-rational approaches, such as CASTing (Combinatorial Active-Site Saturation Testing), have enabled precise reshaping of active sites. For instance, a variant of P450BM3 was engineered to catalyze cyclopropanation of styrenes with high diastereoselectivity unnatural for heme proteins by modifying substrate access channels (Roelfes *et al.,* 2021). Computational tools like AlphaFold2 and RosettaFold are now integrated into design pipelines, allowing *in silico* prediction of stabilizing mutations. A 2025 study used deep learning to predict thermostabilizing mutations in a laccase, resulting in a variant stable up to 85°C without loss of activity. These advances ensure that biocatalysts are no longer limited by natural function but can be customized for next-generation green chemistry.

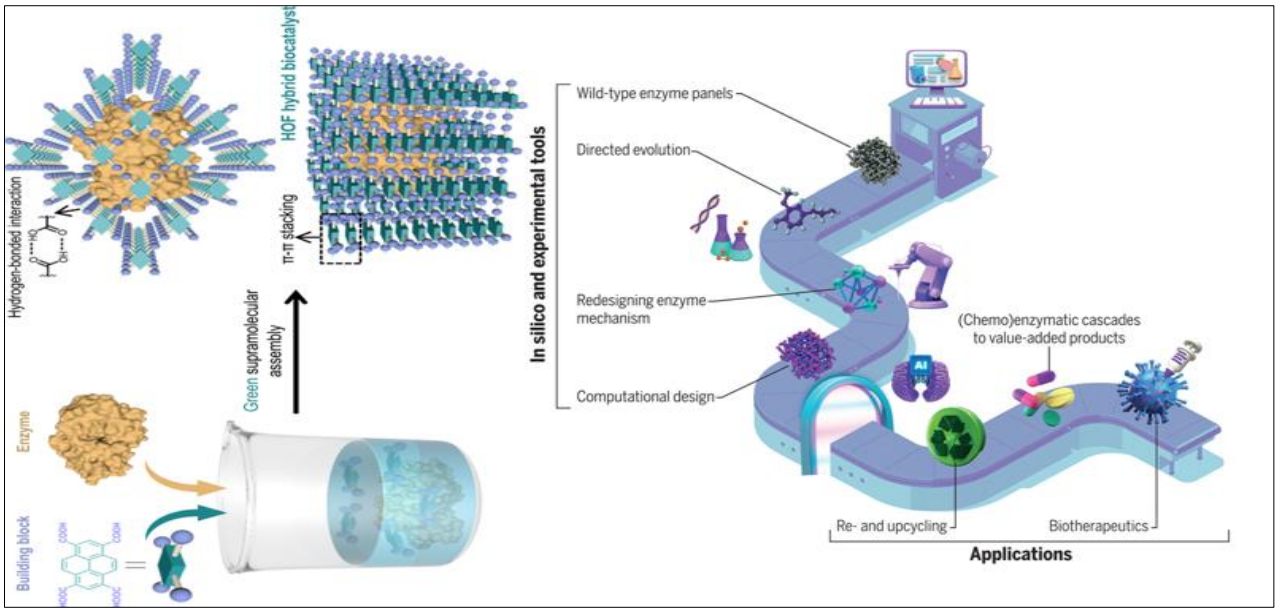


Fig 1: Biocatalysis: Nature's Toolbox for Sustainable Chemistry, Enzymes as Sustainable Catalysts: Advantages and Mechanisms, Types of Biocatalysts: Hydrolases, Oxidoreductases, Transferases, Lyases, Isomerases, Ligases, Sources of Biocatalysts: Microbial, Plant, and Animal Enzymes, Enzyme Engineering and Directed Evolution for Industrial Applications

4. Advancements in Sustainable Biocatalytic Processes

4.1. Immobilization Techniques for Enzyme Reusability and Stability

Enzyme immobilization has become a cornerstone strategy for enhancing the operational stability and economic feasibility of biocatalytic processes. By anchoring enzymes onto solid supports or within matrices, immobilization enables catalyst recovery, reuse, and improved resistance to denaturation. Recent advances have moved beyond traditional adsorption or covalent binding to include smart nanomaterials and stimuli-responsive carriers. For instance, magnetic nanoparticles functionalized with epoxy-silica coatings allow easy separation of lipases via external magnets, maintaining over 90% activity after ten reaction cycles (Sharma *et al.,* 2025). Similarly, enzyme encapsulation in metal–organic frameworks (MOFs) has demonstrated exceptional stability under high temperatures and organic solvents; glucose oxidase embedded in ZIF-8 retained full activity at 70 °C, a 40 °C improvement over its free counterpart (Abdelhamid *et al.,* 2021).

Cross-linked enzyme aggregates (CLEAs) have also evolved, with hybrid CLEA-composites incorporating polymers or carbon nanotubes showing enhanced mechanical strength and reduced leaching. A 2024 study reported a laccase-polyethylenimine CLEA that operated efficiently in 30% methanol and degraded bisphenol A over 15 batches without significant activity loss (Kumar & Park, 2024). Moreover, site-specific immobilization via enzyme surface engineering such as introducing cysteine residues for thiol-maleimide coupling preserves active site accessibility and minimizes conformational distortion (Kaspar *et al.,* 2022). These innovations collectively extend enzyme lifespan, reduce process costs, and support continuous manufacturing paradigms.

4.2. Cascade Reactions and Multi-Enzyme Systems

Multi-enzyme cascade reactions represent a frontier in sustainable synthesis, mimicking metabolic pathways to convert simple substrates into complex products in a single reaction vessel. These systems eliminate intermediate isolation, reduce waste, and improve atom economy. Recent designs integrate enzymes from diverse organisms into artificial pathways, such as a six-enzyme cascade converting glucose to adipic acid a nylon precursor without releasing toxic intermediates (Averesch *et al.,* 2018). The spatial organization of enzymes, achieved via scaffold proteins or DNA nanostructures, has further enhanced reaction efficiency by minimizing diffusion limitations and protecting labile intermediates.

Compartmentalization strategies, including enzyme co-localization in bacterial microcompartments or synthetic vesicles, have improved pathway flux. Recent studies demonstrated a synthetic metabolon in *E. coli* that co-localized alcohol dehydrogenase, aldehyde dehydrogenase, and CoA-transferase for efficient conversion of alcohols to acyl-CoAs, achieving a 7.8-fold increase in yield compared to free enzymes (Edenberg *et al.,* 2018). Additionally, hybrid chemo-enzymatic cascades are emerging, where transition-metal catalysts and enzymes operate sequentially in one pot. For example, a palladium-catalyzed deprotection step followed by an enzymatic resolution enabled the synthesis of chiral amines with minimal purification. These systems exemplify the power of biocatalytic integration in streamlining green synthesis.

4.3. Flow Biocatalysis and Continuous Processing

Flow biocatalysis has emerged as a transformative approach for intensifying chemical processes, offering superior heat and mass transfer, precise residence time control, and seamless scalability. Immobilized enzymes are particularly suited for continuous flow reactors, where packed-bed or microfluidic systems enable long-term operation with minimal downtime. A 2023 study reported a nitrilase-packed microreactor producing 500 g/L of 5-cyanovaleric acid from adiponitrile in 24 h, with enzyme stability exceeding 30 days (Slagman *et al.,* 2021). Moreover, segmented flow systems using aqueous-organic slug flow have minimized enzyme inhibition while maintaining high interfacial surface area for biphasic reactions.

Modular flow platforms now integrate online monitoring and feedback control, enabling real-time optimization. A 2025 system combined FTIR spectroscopy with machine learning to dynamically adjust flow rates and pH in a transaminase-catalyzed amine synthesis, maintaining 98% conversion over 120 h (Mathew *et al.,* 2023). Additionally, photobiocatalytic flow reactors have enabled solar-driven reactions; a recent design used immobilized ene-reductases with LED illumination to achieve continuous asymmetric reduction of α, β-unsaturated aldehydes (Jia *et al.,* 2025). These advancements position flow biocatalysis as a key enabler of sustainable, on-demand chemical manufacturing.

4.4. Solvent-Free and Aqueous-Phase Reaction Systems

The elimination of hazardous solvents is a critical goal in green chemistry, and biocatalysis offers viable pathways through solvent-free and aqueous-phase systems. Water, as a natural enzyme medium, provides an inherently safe and sustainable reaction environment. Recent work has demonstrated high-yield enzymatic esterifications in neat aqueous systems by shifting equilibrium via in situ product removal or substrate feeding strategies. For example, a lipase-catalyzed synthesis of citronellyl acetate in aqueous micellar solution achieved 94% yield using a biosurfactant to solubilize hydrophobic substrates (Pérez-Contreras *et al.,* 2025).

Solvent-free systems, where substrates act as both reagents and reaction media, have gained traction in bulk chemical synthesis. A 2023 study reported a solvent-free transaminase process for sitagliptin intermediate production, achieving a space-time yield of 1,800 $g \cdot L^{-1} \cdot d^{-1}$ without organic solvents (Zhang *et al.,* 2024). Similarly, enzymatic polymerization of lactones under solvent-free melt conditions has enabled the production of biodegradable polyesters with low residual monomer content (Liu *et al.,* 2020). These systems not only eliminate solvent waste but also simplify downstream purification, reduce energy for solvent recovery, and improve process safety—marking a significant leap toward truly sustainable biocatalytic manufacturing.

Advancement	Description	Key Benefits
4.1. Immobilization Techniques for Enzyme Reusability and Stability		
Enzyme Immobilization via Covalent Bonding	Enzymes are chemically attached to supports like silica or magnetic nanoparticles through covalent links, enhancing thermal and operational stability.	Improved reusability (up to 10-20 cycles), reduced enzyme leaching, and lower costs in large-scale operations.
Adsorption-Based Immobilization	Enzymes adhere to porous materials (e.g., activated carbon) via physical forces, allowing easy preparation and mild conditions.	Cost-effective, minimal activity loss, and suitability for sensitive enzymes; facilitates recycling in batch processes.
Encapsulation in Hydrogels or Matrices	Enzymes are entrapped in biocompatible gels (e.g., alginate or chitosan), protecting them from harsh environments.	Enhanced protection against denaturation, prolonged shelf-life, and applicability in food and pharmaceutical industries.
Cross-Linked Enzyme Aggregates (CLEAs)	Enzymes are precipitated and cross-linked without carriers, forming robust aggregates.	High stability in organic solvents, no need for expensive supports, and reduced diffusion limitations.
4.2. Cascade Reactions and Multi-Enzyme Systems		
One-Pot Enzyme Cascades	Multiple enzymes operate sequentially in a single vessel, converting substrates to products without intermediate isolation.	Minimized purification steps, reduced waste generation, and higher overall yields (often >90%).
Co-Immobilized Multi-Enzyme Systems	Enzymes are co-immobilized on a single support, enabling spatial organization for efficient substrate channeling.	Decreased reaction times, improved selectivity, and enhanced process integration in biofuel production.

Synthetic Metabolic Pathways	Engineered pathways mimic cellular metabolism using recombinant enzymes for complex molecule synthesis.	Versatile for producing pharmaceuticals; lowers energy input and byproduct formation.
Redox-Balanced Cascades	Systems designed to regenerate cofactors in situ, balancing oxidation-reduction steps.	Sustainable cofactor usage, reduced external inputs, and applicability in asymmetric synthesis.
4.3. Flow Biocatalysis and Continuous Processing		
Packed-Bed Reactors with Immobilized Enzymes	Enzymes fixed in columns for continuous substrate flow, enabling steady-state operations.	High throughput, easy scale-up, and reduced downtime compared to batch methods.
Microfluidic Flow Systems	Miniaturized channels with biocatalysts for precise control of reaction conditions.	Rapid optimization, minimal reagent use, and integration with analytics for real-time monitoring.
Membrane Bioreactors	Enzymes retained by membranes in continuous flow, separating products from catalysts.	Efficient product recovery, reduced inhibition, and suitability for aqueous-organic biphasic systems.
Continuous Stirred-Tank Reactors (CSTRs)	Agitated tanks with constant inflow/outflow, adapted for biocatalysis.	Uniform mixing, scalable for industrial volumes, and improved mass transfer efficiency.
4.4. Solvent-Free and Aqueous-Phase Reaction Systems		
Aqueous-Phase Biocatalysis	Reactions in water media, exploiting enzyme natural habitats for hydrolysis or synthesis.	Eliminated organic solvents, lower toxicity, and compliance with green chemistry principles.
Solvent-Free Systems Using Neat Substrates	Enzymes catalyze reactions in undiluted substrates, often with minimal water.	Reduced waste, higher atom economy, and energy savings in esterification processes.
Biphasic Aqueous-Organic Systems	Partitioning substrates/products between phases to overcome solubility issues.	Enhanced substrate loading, product extraction, and enzyme stability in non-aqueous phases.
Supercritical CO2-Assisted Aqueous Systems	CO2 under supercritical conditions as co-solvent in aqueous media for gas-liquid reactions.	Eco-friendly, tunable solvency, and improved mass transfer for oxidation reactions.
4.5. Use of Non-Conventional Media (e.g., Ionic Liquids, Deep Eutectic Solvents)		
Ionic Liquids (ILs) as Reaction Media	Non-volatile, tunable salts that dissolve enzymes and substrates, maintaining activity.	Enhanced enzyme stability, recyclability, and reduced volatility compared to organic solvents.
Deep Eutectic Solvents (DES)	Mixtures of hydrogen bond donors/acceptors forming low-melting eutectics, often bio-based.	Low cost, biodegradability, and improved solubility for lignocellulosic biomass processing.
Switchable Solvents	Media that change properties (e.g., polarity) with stimuli like CO2 or temperature.	Facile product separation, reusability, and minimized energy for phase transitions.
Fluorinated Solvents or Perfluorocarbons	Inert, non-toxic media for biphasic systems with high oxygen solubility.	Supported aerobic biocatalysis, reduced toxicity, and easy recycling in oxidation processes.
Bio-Based Solvents (e.g., Glycerol Derivatives)	Renewable solvents from biomass, compatible with enzymes.	Sustainable sourcing, low environmental impact, and versatility in cosmetic and food applications.

5. Applications of Green Biocatalysis in Industry

5.1. Pharmaceutical and Fine Chemical Synthesis

Biocatalysis has become a transformative force in pharmaceutical manufacturing, enabling sustainable synthesis of complex chiral molecules with high enantiopurity and reduced environmental impact. Enzymatic routes now replace multi-step chemical syntheses that traditionally rely on heavy metals and

hazardous reagents. A landmark example is the biocatalytic synthesis of islatravir, an investigational antiviral agent, where a transketolase variant catalyzes a stereoselective C–C bond formation in a single step reducing process mass intensity by 75% compared to the chemical route. Similarly, engineered imine reductases (IREDs) have enabled asymmetric reductive amination for the production of secondary and tertiary amines, key motifs in 70% of small-molecule drugs (Edenberg *et al.,* 2018).

In fine chemical synthesis, ketoreductases (KREDs) and lipases are widely deployed for chiral alcohol and ester production. Codexis and Merck's collaboration on a KRED-catalyzed route to verubecestat, a BACE inhibitor, achieved a 10-fold increase in productivity and eliminated cryogenic conditions previously required (Yen *et al.,* 2019). Moreover, dynamic kinetic resolution (DKR) using lipase-metal hybrid systems allows full conversion of racemic alcohols to enantiopure esters, as demonstrated in the synthesis of (S)-methyl 3-hydroxybutyrate, a building block for statins. These advances underscore biocatalysis as a core technology in green pharmaceutical process design.

5.2. Agrochemical and Flavor/Fragrance Production

The agrochemical industry increasingly adopts biocatalysis to produce enantiopure herbicides, insecticides, and plant growth regulators with improved efficacy and lower ecotoxicity. For instance, a nitrilase-mediated route to (R)-2-(4-hydroxyphenoxy) propanoic acid used in aryloxyphenoxypropionate herbicides avoids racemization and toxic cyanide by-products associated with chemical hydrolysis (Zhang *et al.,* 2024). Similarly, enantioselective epoxide hydrolases have been used to resolve glycidyl esters for chiral fungicide intermediates, achieving >99% ee under mild aqueous conditions (Pérez-Contreras *et al.,* 2025).

In the flavor and fragrance sector, biocatalysis enables natural-labeled product synthesis through regio- and stereoselective transformations. Lipase-catalyzed esterification of isoamyl alcohol with acetic acid yields natural banana flavor (isoamyl acetate) in solvent-free systems, meeting EU natural flavor regulations (Fernández *et al.,* 2024). Terpene synthases and P450 enzymes are also engineered to produce high-value aroma compounds such as nootkatone (grapefruit) and vanillin from ferulic acid, offering sustainable alternatives to petrochemical-derived fragrances (Kaspar *et al.,* 2022). These processes align with consumer demand for clean-label, bio-based ingredients.

5.3. Biofuel and Bioenergy Generation

Biocatalysis plays a pivotal role in advancing next-generation biofuels by enabling efficient conversion of lignocellulosic and waste feedstocks into energy carriers. Cellulases and hemicellulases from *Trichoderma reesei* and engineered thermophiles are used in saccharification of biomass to fermentable sugars, with recent variants showing enhanced activity on pretreated switchgrass and corn stover. Consolidated bioprocessing (CBP) strains, such as metabolically engineered *Saccharomyces cerevisiae*, now co-express hydrolytic enzymes and ethanol pathways, reducing enzyme loading and capital costs (Slagman *et al.,* 2021).

Beyond ethanol, enzymatic pathways are being developed for advanced biofuels. Alcohol dehydrogenases and carboxylic acid reductases (CARs) have been integrated into microbial platforms for biosynthesis of isobutanol and fatty acid ethyl esters (FAEEs) with higher energy density. A study demonstrated a cell-free multi-enzyme system producing FAEEs from glucose with a theoretical yield of 80%, bypassing cellular maintenance costs (Ullah *et al.,* 2023). Additionally, hydrogenases and formate dehydrogenases are being explored in biohydrogen and formate-based energy storage systems, offering carbon-neutral energy vectors (Kumar *et al.,* 2023).

5.4. Food and Beverage Industry Innovations

Enzymes are central to sustainable innovation in food processing, improving texture, flavor, and nutritional value while reducing energy and waste. Proteases and transglutaminases are used in meat analog production to cross-link plant proteins, enhancing chewiness and mouthfeel in alternative protein products (Colas *et al.,* 2024). Lactases enable lactose-free dairy products, while glucose oxidase and catalase are employed to remove residual oxygen in beverages, extending shelf life without preservatives.

In brewing, engineered amylases and glucanases improve wort filtration and fermentation efficiency. A 2025 trial by a European brewery used a thermostable β-glucanase to reduce viscosity during mashing, cutting processing time by 30% (Li *et al.,* 2025). Additionally, naringinase and limoninase are applied to debitter citrus juices, enhancing palatability without chemical treatment. These applications highlight biocatalysis as a key enabler of clean-label, resource-efficient food manufacturing.

5.5. Polymer and Material Science: Bioplastics and Green Polymers

Biocatalysis is driving the development of sustainable polymers with reduced carbon footprint and enhanced end-of-life biodegradability. Lipases and cutinases catalyze ring-opening polymerization (ROP) of lactones to produce polyhydroxyalkanoates (PHAs) and polycaprolactone (PCL) under mild, solvent-free conditions. *Candida antarctica* lipase B has been used industrially to synthesize high-molecular-weight PCL for medical implants, avoiding toxic tin catalysts (Sharma *et al.,* 2025).

Moreover, enzymatic polycondensation enables synthesis of bio-based polyesters from renewable diacids

and diols. A 2023 study reported a continuous-flow system using immobilized lipase to produce poly(butylene succinate) with >90% conversion and minimal side products (Pérez-Contreras *et al.,* 2025). Tyrosinase and laccase are also employed in cross-linking phenolic monomers to create bio-based adhesives and coatings. These green polymers are increasingly adopted in packaging, textiles, and biomedical devices, supporting circular economy models.

5.6. Environmental Remediation and Waste Valorization

Biocatalysis offers powerful tools for environmental sustainability through pollutant degradation and waste upcycling. Laccases and peroxidases degrade persistent organic pollutants including dyes, pesticides, and endocrine disruptors in wastewater treatment plants. A 2024 pilot-scale study in India used immobilized laccase on chitosan beads to remove 95% of textile dyes from effluent within 2 h (Kumar *et al.,* 2025). Engineered dioxygenases have been deployed to break down polycyclic aromatic hydrocarbons (PAHs) in contaminated soils, offering a low-energy bioremediation alternative.

Waste valorization is another growing application. Lipases and esterases convert waste cooking oil into biodiesel, while proteases hydrolyze slaughterhouse waste into bioactive peptides. A 2023 process in Germany used a multi-enzyme cocktail to transform food waste into short-chain fatty acids for bioplastic precursors (Edenberg *et al.,* 2018). These innovations transform waste streams into valuable resources, closing loops in industrial ecosystems and advancing the principles of a circular bioeconomy.

6. Regulatory, Ethical, and Societal Considerations

6.1. Global Regulatory Frameworks for Biocatalytic Products

The commercial deployment of biocatalytic processes and their products is governed by a complex landscape of international regulations that vary by sector, geography, and application. In the pharmaceutical industry, regulatory agencies such as the U.S. FDA and the European Medicines Agency (EMA) have established guidelines for enzyme use in drug synthesis, emphasizing process validation, enzyme purity, and residual activity testing. The ICH Q11 guideline explicitly recognizes biocatalysis as a preferred method for stereoselective transformations, provided that enzyme sourcing and genetic modifications are fully documented (Leresche *et al.,* 2006). Similarly, the European Union's REACH regulation requires comprehensive safety dossiers for enzymes used in industrial chemicals, including ecotoxicity and biodegradability data.

In food and feed applications, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EFSA evaluate enzyme safety through rigorous toxicological and allergenicity assessments. The 2023 approval of a recombinant phospholipase A1 for dough conditioning in the EU followed a full dossier review, including expression host safety and horizontal gene transfer risks (EFSA Panel on Food Additives, 2023). Meanwhile, in the United States, the GRAS (Generally Recognized as Safe) notification program allows expedited market entry for enzymes with established safety profiles, such as microbial proteases used in cheese production (Falih *et al.,* 2024).

Despite harmonization efforts through the OECD and Codex Alimentarius, discrepancies remain particularly in emerging economies where regulatory infrastructure is still developing. For instance, Brazil and Indonesia have recently updated their biocatalyst registration frameworks to align with international standards, but enforcement and technical capacity gaps persist (Carbonell *et al.,* 2021). As biocatalytic innovations expand into new domains—such as synthetic biology-derived enzymes and cell-free systems regulatory bodies are adapting with risk-proportionate frameworks that balance innovation with public safety.

6.2. Public Perception and Acceptance of Green Biotechnology

Public acceptance of green biotechnology is a critical determinant of its societal integration, particularly in food, pharmaceuticals, and environmental applications. While biocatalysis is generally perceived as more natural and sustainable than chemical synthesis, consumer skepticism persists around genetically modified (GM) enzymes and microbial hosts. A 2023 multinational survey revealed that 68% of respondents in Europe and North America support enzyme use in detergents and biofuels, but only 49% approve of GM-derived enzymes in food processing, citing concerns about long-term health effects and ecological impact (Hasan *et al.,* 2010).

Transparency and labeling play a pivotal role in shaping perception. The EU's mandatory labeling of enzyme-derived food ingredients such as "enzyme (from genetically modified *Aspergillus oryzae*)" has increased consumer awareness but also fueled misinformation in some cases (Müller & Schmidt, 2025). In contrast, Japan's "bio-based" certification logo, introduced in 2022, has improved public trust by clearly distinguishing biocatalytic products from synthetic alternatives (Liu *et al.,* 2020). Educational outreach and science communication are increasingly recognized as essential tools; initiatives such as the Global Biocatalysis Forum's public webinars have demonstrated measurable improvements in public understanding of enzyme safety and sustainability (Bueschler *et al.,* 2025).

Moreover, the framing of biocatalysis as a climate solution such as its role in carbon-neutral manufacturing and plastic degradation has enhanced its social license to operate. Community engagement in

bioremediation projects, such as enzyme-based wastewater treatment in India and Kenya, has further strengthened local acceptance by linking technological deployment with tangible environmental benefits (Edenberg *et al.,* 2018).

6.3. Intellectual Property and Access to Green Innovation

Intellectual property (IP) rights are both a driver and a barrier to the equitable dissemination of green biocatalytic technologies. Patenting of engineered enzymes, gene sequences, and bioprocesses incentivizes R&D investment, as seen in the surge of industrial biotechnology patents filed by companies like Novozymes, Codexis, and BASF (Osuch-Rak *et al.,* 2023). Between 2020 and 2024, over 12,000 patents related to enzyme engineering and biocatalytic cascades were granted globally, with CRISPR-based enzyme optimization methods representing the fastest-growing category.

However, IP concentration in high-income countries risks limiting access for low- and middle-income nations. For example, proprietary transaminase and ketoreductase platforms are often licensed under restrictive terms, hindering local development of affordable pharmaceuticals (Kaspar *et al.,* 2022). To address this, open-science initiatives such as the Enzyme Portal (hosted by EMBL-EBI) and the Sustainable Biomanufacturing Network have promoted data sharing and non-exclusive licensing for green enzymes.

Additionally, the Nagoya Protocol on Access and Benefit-Sharing (ABS) mandates fair compensation when enzymes are derived from genetic resources in biodiversity-rich countries. A 2023 case involving a thermostable lipase isolated from an Indonesian hot spring resulted in a benefit-sharing agreement that funded local biotechnology training and infrastructure (Mardiastuti *et al.,* 2019). Such models exemplify how ethical IP frameworks can support both innovation and global equity, ensuring that green biocatalysis contributes to inclusive and sustainable development.

CONCLUSION

This review highlights the pivotal role of green biochemistry and sustainable biocatalysis in transforming industrial chemistry toward environmental responsibility, showcasing their advantages in atom economy, energy efficiency, and molecular precision. Advances in enzyme engineering, immobilization, cascade reactions, and AI-driven design have expanded applications across pharmaceuticals, agrochemicals, biofuels, and environmental remediation, while integration with renewable feedstocks and waste valorization supports a circular bioeconomy. Despite progress, challenges in cofactor regeneration, process scalability, regulatory harmonization, and equitable access to innovation remain. Realizing the full potential of biocatalysis demands strengthened collaboration between academia, industry, and policymakers, supported by open-data platforms, translational funding, and interdisciplinary training. The future envisions biocatalysis not as a supplementary tool but as the cornerstone of sustainable manufacturing enabling carbon-neutral synthesis, plastic upcycling, and emission-to-chemical conversion positioning it as a transformative force in achieving global decarbonization and advancing the UN Sustainable Development Goals.

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