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APPLICATION OF GREEN CHEMISTRY AND BIOCONVERSION METHODS FOR SUSTAINABLE PHARMACEUTICAL API AND INTERMEDIATE SYNTHESIS

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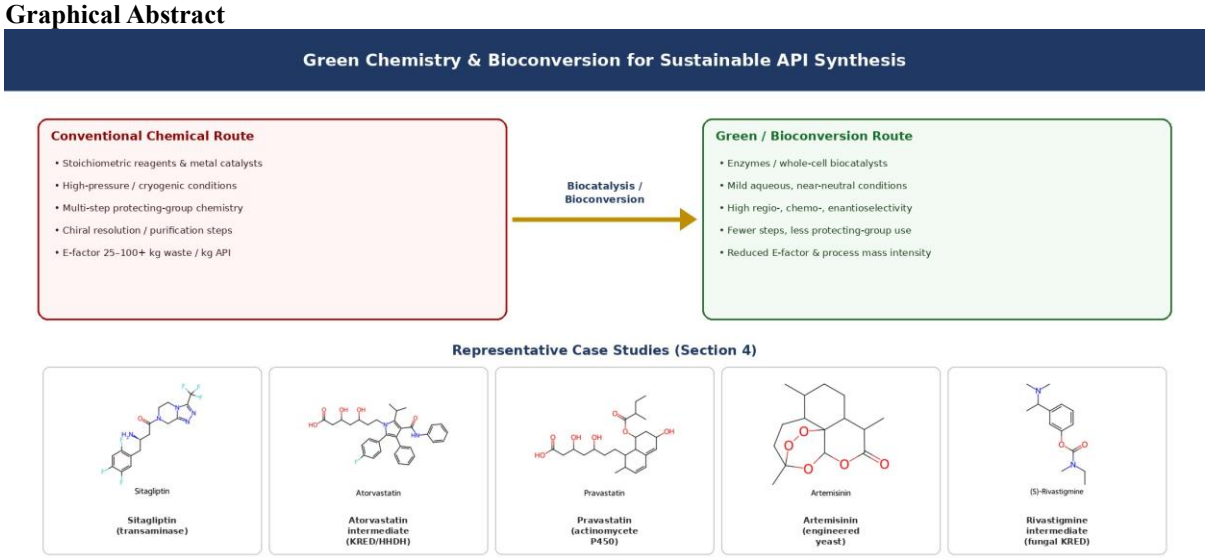
Highlights

- Green chemistry metrics (atom economy, E-factor, process mass intensity) provide a quantitative basis for redesigning pharmaceutical API and intermediate synthesis.
- Biocatalysis and whole-cell bioconversion deliver mild, highly regio-, chemo- and enantioselective alternatives to conventional stoichiometric and metal-catalysed chemistry.
- Five industrially or academically validated case studies are reviewed in depth: sitagliptin (transaminase), the atorvastatin side-chain intermediate (ketoreductase/halohydrin dehalogenase), pravastatin (actinomycete cytochrome P450 hydroxylation), semisynthetic artemisinin (engineered yeast), and a rivastigmine chiral intermediate (locally isolated Fusarium graminearum).
- Directed enzyme evolution and whole-cell fermentation optimisation (pH, temperature, agitation, incubation period) are shown, with real reported data, to be central to converting a laboratory-scale biocatalytic hit into a robust manufacturing process.
- Persisting challenges — enzyme discovery cost, substrate scope, cofactor dependence and regulatory validation — mean biocatalysis is best deployed selectively, at the specific synthetic steps where its selectivity advantage is decisive.

Graphical Abstract



Graphical Abstract. Conventional, waste-intensive chemical synthesis (left) versus enzyme/whole-cell-catalysed green synthesis (right), illustrated by the five biocatalytic case studies reviewed in Section 4: sitagliptin, the atorvastatin side-chain intermediate, pravastatin, semisynthetic artemisinin, and a rivastigmine chiral intermediate.

ABSTRACT

The pharmaceutical industry has historically carried one of the highest environmental (E)factors of any manufacturing sector, generating tens to hundreds of kilograms of waste per kilogram of active pharmaceutical ingredient (API) produced, largely from stoichiometric reagents, protecting-group chemistry, and organic solvent use. Over the past two decades, the twelve principles of green chemistry, together with quantitative greenness metrics such as atom economy, the environmental factor (E-factor) and process mass intensity (PMI), have provided a systematic framework for redesigning API and intermediate synthesis toward greater sustainability. Biocatalysis and microbial bioconversion have emerged as particularly powerful enablers of this redesign, since enzymes and whole-cell biocatalysts operate under mild aqueous conditions, are inherently biodegradable, and offer a level of regio-, chemo- and stereoselectivity that is difficult to match with conventional organic synthesis — an advantage of direct relevance given that most modern APIs are chiral. This review consolidates recent

literature on the application of green chemistry principles and bioconversion methods to pharmaceutical API and intermediate manufacture, examining the underlying enzyme classes (transaminases, ketoreductases, halohydrin dehalogenases, cytochrome P450 monooxygenases and nitrilases), whole-cell microbial hydroxylation and bioreduction, and engineeredmicroorganism platforms. Five case studies are analysed in depth: the transaminase-catalysed asymmetric synthesis of sitagliptin, the ketoreductase/halohydrin dehalogenase cascade used for the atorvastatin side-chain intermediate, the actinomycete-mediated microbial hydroxylation of compactin (mevastatin) to pravastatin, the engineered-yeast route to semisynthetic artemisinin, and the whole-cell fungal bioreduction of a chiral rivastigmine intermediate by a locally isolated strain of Fusarium graminearum, including the reported effects of pH, temperature, incubation period and agitation speed on conversion and enantiomeric excess. Complementary green technologies — solvent selection guides, continuous-flow biocatalysis, and directed enzyme evolution — are also discussed, alongside the persisting technical, economic and regulatory challenges that continue to limit the wider adoption of bioconversion routes at commercial scale. The review concludes by identifying machine-learning-guided enzyme engineering, synthetic biology and standardised green metrics as the most promising directions for the continued sustainable transformation of pharmaceutical manufacturing.

KEYWORDS: Green chemistry; Biocatalysis; Bioconversion; Active pharmaceutical ingredient (API); Sustainable synthesis; Enzyme engineering; E-factor; Fungal bioreduction

1. INTRODUCTION

Active pharmaceutical ingredient (API) manufacturing has long been recognised as one of the least resource-efficient branches of the chemical industry. Multistep syntheses involving stoichiometric reagents, protecting-group manipulations, cryogenic or high-pressure conditions, and large volumes of organic solvent routinely generate between twenty-five and over one hundred kilograms of waste for every kilogram of API produced — an environmental factor (E-factor) far higher than that typically reported for bulk or even fine chemicals, despite the pharmaceutical sector operating at a comparatively small annual production scale (Figure 1). This waste burden, together with growing regulatory, environmental and economic pressure, has driven the pharmaceutical sector to progressively adopt the principles of green chemistry: a design philosophy aimed at reducing or eliminating the use and generation of hazardous substances across the entire life cycle of a chemical product.

Within this broader green chemistry movement, biocatalysis and microbial bioconversion occupy a distinctive and increasingly central position. Enzymes and whole-cell biocatalysts operate under mild, typically aqueous, near-neutral-pH conditions; they are generated from renewable biological sources; they are inherently biodegradable; and, critically for pharmaceutical synthesis, they offer a level of regioselectivity, chemoselectivity and enantioselectivity that is often difficult or costly to achieve using conventional organic catalysts or stoichiometric chiral auxiliaries. Because the great majority of modern small-molecule APIs are chiral, and because a single incorrect stereocentre can render a drug inactive or even toxic, this selectivity advantage translates directly into fewer synthetic steps, less protecting-group chemistry, and substantially reduced waste generation.

This review consolidates recent literature on the application of green chemistry principles and bioconversion methods specifically to the synthesis of pharmaceutical APIs and their key synthetic intermediates. It begins by outlining the twelve principles of green chemistry and the quantitative metrics — atom economy, E-factor and process mass intensity — used to benchmark synthetic routes against these principles. It then surveys the principal classes of enzymes and microbial systems used in industrial bioconversion, before examining five case studies in depth: the transaminase-catalysed manufacture of sitagliptin, the ketoreductase/halohydrin-dehalogenase route to the atorvastatin side-chain intermediate, the actinomycete-mediated bioconversion of compactin (mevastatin) to pravastatin, the engineered-yeast platform for semisynthetic artemisinin, and the whole-cell fungal bioreduction of a chiral rivastigmine intermediate. The review concludes with a discussion of complementary green technologies, the practical challenges that continue to limit wider adoption of bioconversion routes, and the directions most likely to shape the field's continued development.

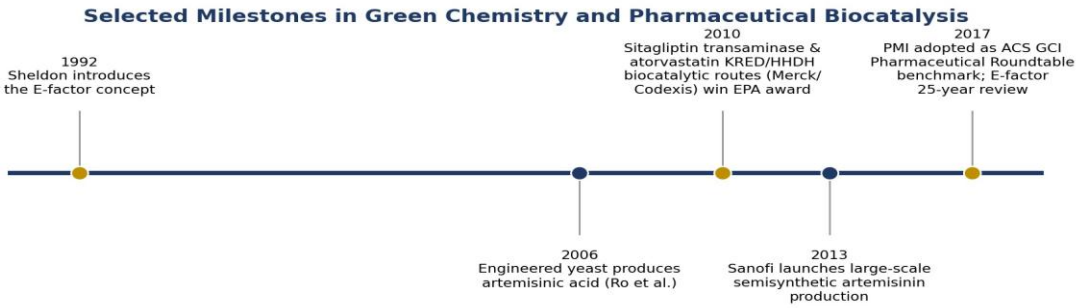


Figure 1. Selected milestones in the development of green chemistry metrics and pharmaceutical biocatalysis, from the introduction of the E-factor concept (1992) through the sitagliptin/atorvastatin biocatalytic process awards (2010) to the large-scale launch of semisynthetic artemisinin (2013).

2. Principles and Metrics of Green Chemistry in Pharmaceutical Manufacturing

2.1 The Twelve Principles of Green Chemistry

The twelve principles of green chemistry, first articulated by Anastas and Warner in the mid1990s, remain the conceptual foundation against which pharmaceutical synthetic routes continue to be evaluated. Applied to API and intermediate manufacture, the most operationally relevant of these principles include: preventing waste rather than treating or disposing of it after the fact; maximising atom economy so that the greatest possible proportion of the atoms in the starting materials are incorporated into the final product; using and generating substances with little or no toxicity; designing energy-efficient processes that avoid unnecessarily high temperatures or pressures; using renewable feedstocks in preference to depleting ones; avoiding unnecessary derivatisation (such as protecting groups) wherever possible; and preferring catalytic, including biocatalytic, reagents over stoichiometric ones. Reviews of contemporary pharmaceutical green chemistry consistently identify solvent selection, catalytic (including enzymatic) methodology, multicomponent and one-pot reactions, continuous processing, and process intensification as the areas of greatest practical impact when these principles are translated into actual manufacturing practice.

2.2 Quantifying Greenness: Atom Economy, E-Factor and Process Mass Intensity

Because qualitative adherence to green chemistry principles is difficult to compare objectively across competing synthetic routes, a small number of quantitative metrics have become the de facto standard for benchmarking greenness in pharmaceutical process development. Atom economy, introduced by Trost, expresses the percentage of the combined molecular weight of the starting materials that is retained in the desired product, providing a purely stoichiometric, reaction-level measure of theoretical efficiency. The environmental factor (E-factor), introduced by Sheldon in 1992, is a practical, mass-based metric defined as the total mass of waste generated (everything produced other than the desired product, including solvents, reagents, catalysts and process aids) divided by the mass of product obtained. As summarised in Figure 2, Sheldon's original industry-sector comparison showed E-factors rising sharply as annual production volume falls and product complexity increases — from below 0.1 for the petrochemical industry (annual production of the order of 10^6–10^8 tonnes), through 1–5 for bulk chemicals and 5–50 for fine chemicals, up to 25 and often well over 100 for the pharmaceutical industry, which typically manufactures only 10 to 10^3 tonnes of any given API per year. Process mass intensity (PMI), adopted and standardised by the American Chemical Society Green Chemistry Institute (ACS GCI) Pharmaceutical Roundtable, is closely related to the E-factor (PMI is numerically equal to the E-factor plus one) and expresses the total mass of all materials used in a process divided by the mass of product obtained, making it a convenient benchmarking tool across the many discrete synthetic steps of a multistep API route. It is increasingly emphasised in the literature that these mass-based metrics, while indispensable for practical process comparison, need to be read alongside life-cycle assessment and economic-viability measures in order to capture the full environmental and commercial sustainability of a given synthetic route — a caveat that applies equally to the bioconversionbased routes discussed in this review.

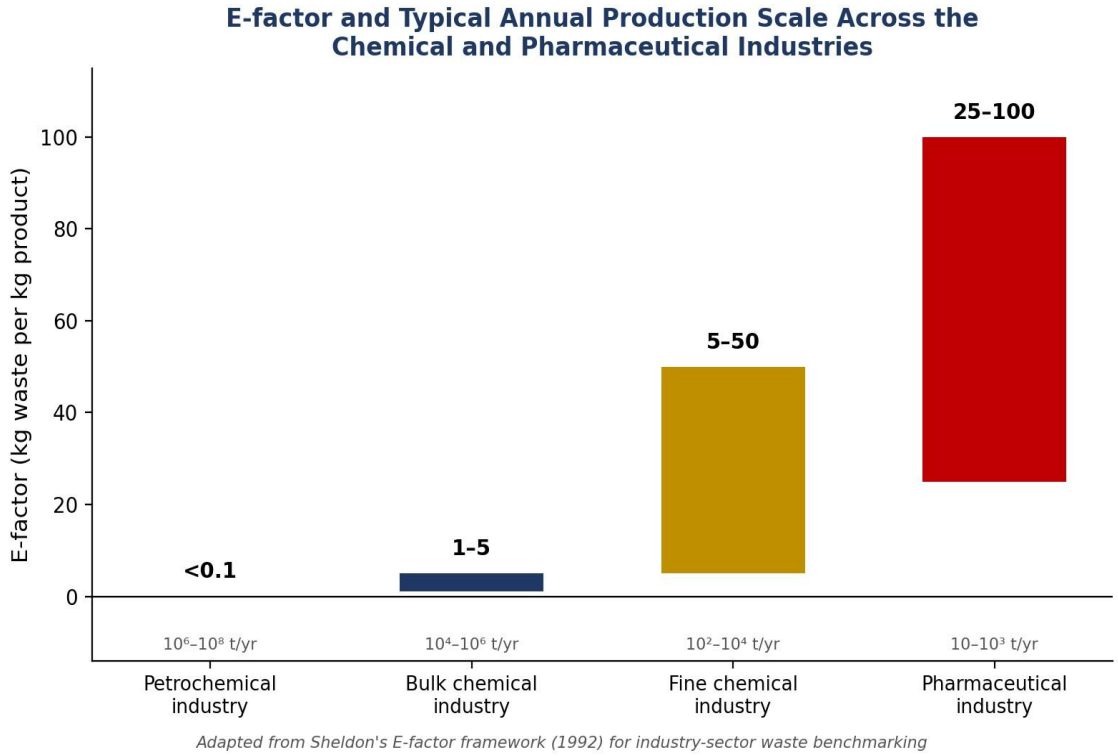


Figure 2. E-factor and typical annual production scale across the petrochemical, bulk chemical, fine chemical and pharmaceutical industries, illustrating why the pharmaceutical sector — despite its comparatively small production volumes — carries the highest reported waste intensity per unit of product.

3. Biocatalysis and Bioconversion as Enablers of Green Pharmaceutical Synthesis

3.1 Enzyme Classes Used in API and Intermediate Synthesis

A relatively small number of enzyme classes account for the great majority of industrially applied pharmaceutical biocatalysis. Ketoreductases (KREDs), together with a cofactorregeneration partner such as glucose dehydrogenase (GDH), catalyse the highly enantioselective reduction of prochiral ketones to chiral alcohols and are widely used to install stereocentres in statin side chains and related intermediates; whole-cell microorganisms possessing native ketoreductase/alcohol dehydrogenase activity, such as the filamentous fungi discussed in Section 4.5, can achieve the same transformation without the need to isolate and purify the enzyme. Transaminases (also called aminotransferases) catalyse the direct, pyridoxal-5′-phosphate-dependent conversion of a prochiral ketone to a chiral primary amine, replacing multistep resolution- or reductive-amination-based routes with a single, highly stereoselective biocatalytic step. Halohydrin dehalogenases (HHDHs) catalyse the ringopening substitution of a halohydrin by a range of nucleophiles (including cyanide), enabling carbon–carbon and carbon–heteroatom bond formation under mild, near-neutral aqueous conditions in place of harsher classical substitution chemistry. Cytochrome P450 monooxygenases catalyse highly regio- and stereoselective C–H hydroxylation reactions that are difficult to replicate with conventional oxidants, and are the key catalytic entities responsible for several important statin bioconversions. Other enzyme classes of established pharmaceutical relevance include lipases and esterases (kinetic resolution and ester chemistry), nitrilases and nitrile hydratases (conversion of nitriles to carboxylic acids or amides), and deoxyribose-phosphate aldolases (DERA) used in carbon–carbon bond-forming aldol cascades for statin side-chain synthesis.

3.2 Whole-Cell Microbial Bioconversion

In addition to isolated-enzyme biocatalysis, many industrially important pharmaceutical transformations are still carried out using whole, living microbial cells rather than purified enzymes, particularly where the reaction requires an intracellular cofactor-regeneration system, a multi-enzyme pathway, or an enzyme (such as certain cytochrome P450 systems) that is more stable and active within its native cellular context. Actinomycetes — filamentous, Grampositive bacteria including the genera Streptomyces, Actinomadura, Nonomuraea and Pseudonocardia — have proved particularly valuable in this respect, being widely screened and industrially exploited for their ability to perform regioselective hydroxylation of complex polyketide-derived molecules such as the statins. Filamentous fungi isolated directly from environmental soil samples represent a complementary and often more accessible source of whole-cell biocatalysts for asymmetric ketone bioreduction, as illustrated by the rivastigmine intermediate case study in Section 4.5, in which a newly isolated strain of Fusarium graminearum was screened, alongside several other locally isolated fungi, for its ability to reduce a prochiral aryl ketone to the corresponding chiral secondary alcohol. Whole-cell bioconversion of this kind typically involves growing the microbial biomass to an appropriate density, then adding the substrate (either during or after growth) to a fermentation or restingcell system, allowing the native or induced enzymatic machinery of the organism to carry out the desired transformation before the product is recovered by extraction and purification.

3.3 Enzyme and Strain Engineering: Directed Evolution and Process Optimisation

A recurring theme across the biocatalytic case studies discussed in Section 4 is that naturally occurring enzymes or wild-type microbial isolates rarely possess, on first identification, sufficient activity, stability or selectivity toward the specific pharmaceutical substrate of interest to be directly useful at manufacturing scale. For isolated-enzyme biocatalysts, directed evolution — iterative rounds of gene mutagenesis (rational, semi-rational or random), expression, and high-throughput screening or selection for the desired improved property — has become the standard engineering approach for converting a marginally active “hit” enzyme into a robust, commercially viable industrial biocatalyst (Figure 3). For whole-cell fungal or actinomycete biocatalysts, an analogous improvement is achieved not by re-engineering the organism's genome but by systematically optimising the fermentation and bioconversion process parameters — pH, temperature, incubation period, agitation speed, carbon source and substrate loading — to maximise conversion and enantiomeric excess, as demonstrated in detail for the rivastigmine intermediate case study in Section 4.5. More recently, the integration of computational protein design and machine-learning-guided variant prioritisation has begun to reduce the number of experimental screening rounds required for isolated-enzyme engineering campaigns, a trend discussed further in Section 8.

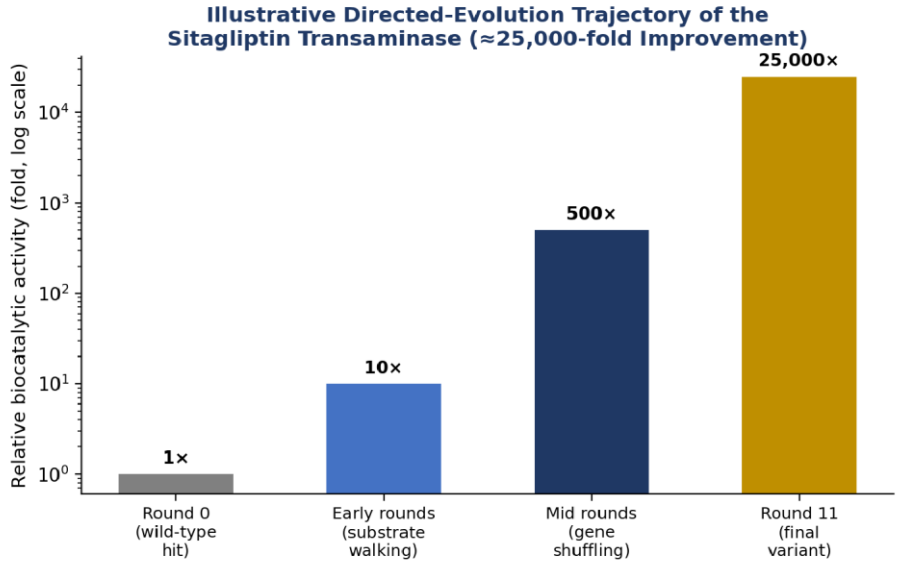


Figure 3. directed-evolution trajectory of the sitagliptin transaminase, showing the compounded, approximately 25,000-fold improvement in biocatalytic activity achieved over multiple rounds of mutagenesis and screening (Section 4.1).

4. Case Studies in Green and Bioconversion-Based API/Intermediate Synthesis

4.1 Sitagliptin: Transaminase-Catalysed Asymmetric Amination

Sitagliptin, the active ingredient of the antidiabetic medicine marketed as Januvia®, was originally manufactured via an asymmetric rhodium-catalysed hydrogenation of an unprotected enamine. Although efficient, this route required a high-pressure hydrogenation step (around 250 psi) with specialised equipment, a rhodium catalyst that had to be removed to very low residual levels, and an additional crystallisation step to enrich the stereoisomeric purity of the product. Scientists at Merck and the enzyme-engineering company Codexis screened available transaminase enzymes for activity toward the prositagliptin ketone and found no naturally occurring enzyme with any detectable activity. Using a substrate-walking and structure-guided mutagenesis strategy followed by more than ten rounds of directed evolution and some twenty-seven individual mutations, the collaboration produced an engineered (R)-selective transaminase with a greater than 25,000-fold improvement in biocatalytic activity relative to the starting enzyme, capable of converting the ketone directly to sitagliptin in a single step with no detectable formation of the undesired enantiomer. The resulting biocatalytic process eliminated the high-pressure hydrogenation, removed the need for rhodium and other metal catalysts entirely, and eliminated the chiral-purification step, delivering a reported 56% improvement in manufacturing productivity, a 10–13% increase in overall yield, and a 19% reduction in total waste generation. The work, published jointly by Merck and Codexis scientists, was recognised with the United States Environmental Protection Agency's Presidential Green Chemistry Challenge Award in 2010.

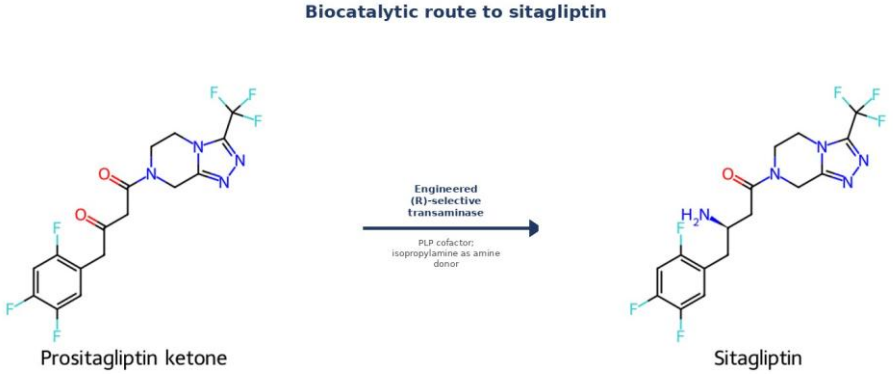


Figure 4. Biocatalytic route to sitagliptin: an engineered (R)-selective transaminase converts the prositagliptin ketone directly to sitagliptin in a single asymmetric step, using isopropylamine as the amine donor.

4.2 Atorvastatin Side-Chain Intermediate: Ketoreductase/Halohydrin Dehalogenase Cascade

Atorvastatin, marketed as Lipitor®, has for many years been among the best-selling drugs in the world, making the efficiency of its manufacture a matter of substantial commercial as well as environmental interest. A key chiral building block used in most synthetic routes to atorvastatin is a 3,5-dihydroxy-hexanoate-type side chain bearing two defined stereocentres. Researchers at Codexis, working with Delft University of Technology, developed a “green-bydesign” two-step, three-enzyme biocatalytic process for this intermediate. In the first step, a ketoreductase (KRED), coupled with glucose and an NADP-dependent glucose dehydrogenase (GDH) for in

situ cofactor regeneration, reduces ethyl-4-chloroacetoacetate to the corresponding (S)-ethyl-4-chloro-3-hydroxybutyrate in 96% isolated yield and greater than 99.5% enantiomeric excess. In the second step, a halohydrin dehalogenase (HHDH) catalyses substitution of the chloro group with cyanide at neutral pH and ambient temperature, avoiding the strongly basic, cryogenic Claisen-condensation-type chemistry (historically requiring lithium diisopropylamide at −78 °C) used in earlier synthetic routes. As with the sitagliptin case, the naturally occurring KRED and HHDH enzymes initially displayed selectivity but insufficient productivity for commercial application, and were substantially improved through in vitro directed evolution (gene shuffling) and, in subsequent work, focused directed evolution specifically targeting the halohydrin dehalogenase, to meet predefined manufacturing performance criteria. This case study is particularly instructive because the engineered ketoreductase platform developed for the atorvastatin intermediate was subsequently adapted for use in the manufacture of unrelated APIs, including montelukast and duloxetine.

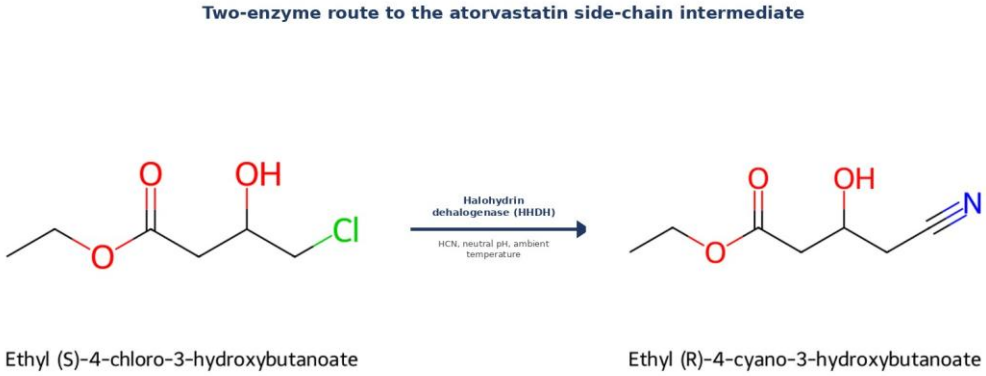


Figure 5. Two-enzyme route to the atorvastatin side-chain intermediate: ketoreductase-mediated asymmetric reduction followed by halohydrin-dehalogenase-catalysed cyanide substitution.

4.3 Pravastatin: Microbial Hydroxylation of Compactin (Mevastatin) by Actinomycetes

Pravastatin, an HMG-CoA reductase inhibitor used for lowering serum cholesterol, is industrially manufactured by a two-stage fermentation/bioconversion process rather than by de novo chemical synthesis. In the first stage, the fungus Penicillium citrinum is fermented to produce compactin (also known as mevastatin or ML-236B); in the second stage, compactin undergoes a highly regioselective 6β-hydroxylation to yield pravastatin. This hydroxylation step has historically been the more challenging of the two and has been the subject of extensive microbial screening, since the desired product differs from several undesired hydroxylation regiochemistries (such as the 3α- and 3β-hydroxycompactin products formed by some fungi) by only the position of a single hydroxyl group. Streptomyces carbophilus was among the first organisms shown to carry out this transformation efficiently, using an inducible cytochrome P450 system (designated cytochrome P450sca) that requires NADPH as a cofactor. Subsequent screening programmes identified additional actinomycete strains capable of the same bioconversion, including Actinomadura sp. ATCC 55678 (which achieved 65–78% conversion of added compactin depending on substrate concentration and incubation time, via a cell-free hydroxylase requiring NADPH and Mg^{2+}), Streptomyces sp. Y-110, Nonomuraea recticatena, and engineered strains of Pseudonocardia autotrophica carrying a heterologous expression vector for improved biotransformation efficiency; a cytochrome P450 (CYP105D7) from Streptomyces avermitilis capable of the same 6-hydroxylation has also been structurally and kinetically characterised. Microbial bioconversion of this kind avoids the need for a multistep, low-temperature, protecting-group-dependent chemical hydroxylation of a structurally complex, sterically hindered polyketide substrate.

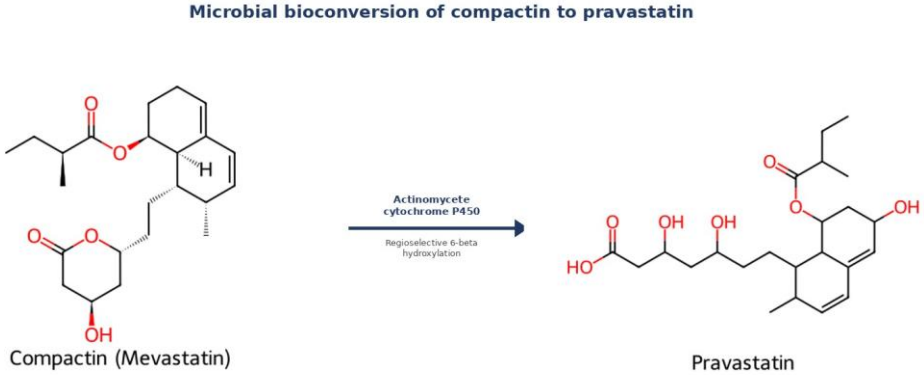


Figure 6. Microbial bioconversion of compactin to pravastatin: actinomycete cytochrome P450 systems catalyse a highly regioselective 6β-hydroxylation.

4.4 Semisynthetic Artemisinin: Engineered-Yeast Production of Artemisinic Acid

Artemisinin, a sesquiterpene lactone endoperoxide extracted from the plant Artemisia annua (sweet wormwood), is the key component of artemisinin-based combination therapies, the World Health Organization's recommended first-line treatment for uncomplicated Plasmodium falciparum malaria. Because plant-derived artemisinin supply is subject to a roughly fourteenmonth agricultural production cycle and consequently large price and availability fluctuations, a synthetic-biology-based alternative supply route was pursued over more than a decade of academic and industrial research. Researchers led by Jay Keasling at the University of California, Berkeley, first engineered the yeast Saccharomyces cerevisiae to express a cytochrome P450 monooxygenase (CYP71AV1) and associated reductase from A. annua, redirecting the yeast's native mevalonate/isoprenoid pathway to produce the artemisinin precursor artemisinic acid. Subsequent work by scientists at Amyris Biotechnologies, published in Nature in 2013, reported a substantially re-engineered yeast strain capable of producing artemisinic acid at industrially relevant titres, together with a practical, scalable chemical process for converting the fermented artemisinic acid into artemisinin itself. The pharmaceutical company Sanofi subsequently licensed this platform and began large-scale production of semisynthetic artemisinin at a dedicated facility in Garessio, Italy, operated on a no-profit-no-loss basis to help stabilise the price and supply of this essential antimalarial for low-income countries.

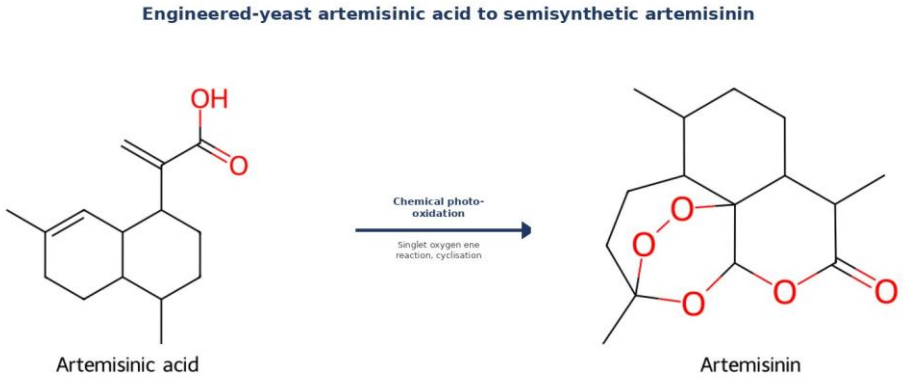


Figure 7. Engineered-yeast route to semisynthetic artemisinin: fermentative production of artemisinic acid from glucose, followed by a chemical photo-oxidation step to complete the endoperoxide-containing artemisinin structure.

4.5 Rivastigmine Intermediate: Whole-Cell Fungal Bioreduction by a Locally Isolated Fusarium graminearum

Rivastigmine, chemically (S)-3-[1-(dimethylamino)ethyl]phenyl N-ethyl-N-methylcarbamate, is a carbamate-class acetylcholinesterase/butyrylcholinesterase inhibitor used in the treatment of mild-to-moderate Alzheimer's disease. The pharmacologically active (S)-enantiomer is conventionally accessed via a chiral secondary alcohol intermediate, N-ethyl-Nmethylcarbamic acid-3-(1S-hydroxyethyl)-phenyl ester, obtained by asymmetric reduction of the corresponding prochiral aryl ketone, N-ethyl-N-methylcarbamoyl acetophenone. A recent study by Basingi and Matadh, published in Current Green Chemistry (2023), reported a chemoenzymatic route to this key intermediate using a soil-derived fungal isolate rather than a purified enzyme or a chemical asymmetric reduction, illustrating that whole-cell fungal bioconversion remains a practical, low-technology entry point into biocatalytic APIintermediate synthesis for laboratories without access to isolated-enzyme engineering platforms. Soil samples collected from six locations in Karnataka, India — including agricultural fields, chemical- and sugar-industry effluent treatment areas, and water-reservoir sites — were processed by the soil dilution plate technique, yielding fungal isolates predominantly belonging to the genera Penicillium, Aspergillus and Fusarium. These isolates, together with several previously reported fungal and yeast biocatalysts (Rhodotorula glutinis, Candida tropicalis PBR-2, Trichothecium roseum, Candida zeylanoides and the marine fungus Penicillium miczynskii), were screened for their ability to reduce acetophenone and its derivatives to the corresponding chiral secondary alcohols (Figure 8); conversion and enantiomeric excess varied considerably between isolates, from 41–97% conversion at 48–99% ee for Candida zeylanoides up to 100% conversion at greater than 99% ee for Rhodotorula glutinis, underscoring the importance of empirical screening across a genetically diverse microbial panel when selecting a whole-cell biocatalyst for a new substrate. Among the isolates obtained specifically from the Karnataka soil samples, Fusarium graminearum was selected for further reaction optimisation and gram-scale conversion of the N-ethyl-N-methylcarbamoyl acetophenone ketone substrate.

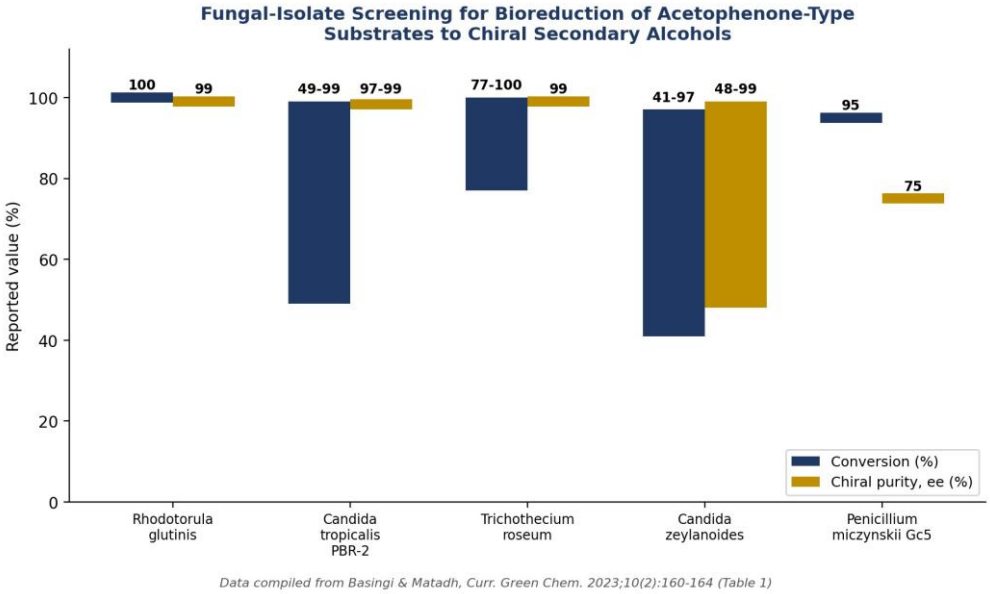


Figure 8. Comparative performance of previously reported fungal and yeast whole-cell biocatalysts for the bioreduction of acetophenone-type substrates to chiral secondary alcohols, providing benchmarking context for the Fusarium graminearum isolate selected in the rivastigmine intermediate study.

The bioreduction process was systematically optimised with respect to pH (4–7.5), temperature (20–40 °C), incubation period (24–96 h) and agitation speed (100–250 rpm), using a ketosubstrate loading of 0.5–1 g L^{-1} in 24-hour-grown culture flasks (Figure 9). Conversion increased with pH up to pH 6 (70% conversion, 98% ee at 24 h) before declining at higher pH, while enantiomeric excess remained close to 96–98% across the entire pH range tested except at the extremes. Temperature showed a similar optimum at 30 °C (81% conversion at 24 h), with a slight decline in ee above 35 °C. Extending the incubation period from 24 to 48 hours increased conversion from 79% to 96% with no loss of enantiomeric excess (98% ee at both time points), but a further extension to 96 hours caused a sharp drop in ee, from above 90% to 77%, without a corresponding gain in conversion — identifying 48 hours as the optimal reaction time. Agitation speed had comparatively little effect on conversion or chirality between 100 and 200 rpm, but a further increase to 250 rpm reduced enantiomeric excess from 98% to 96%. On the basis of this optimisation, fermentative conditions of 48 hours, 30 °C, pH 6 and 200 rpm agitation were identified as optimal for the overall bioreduction process.

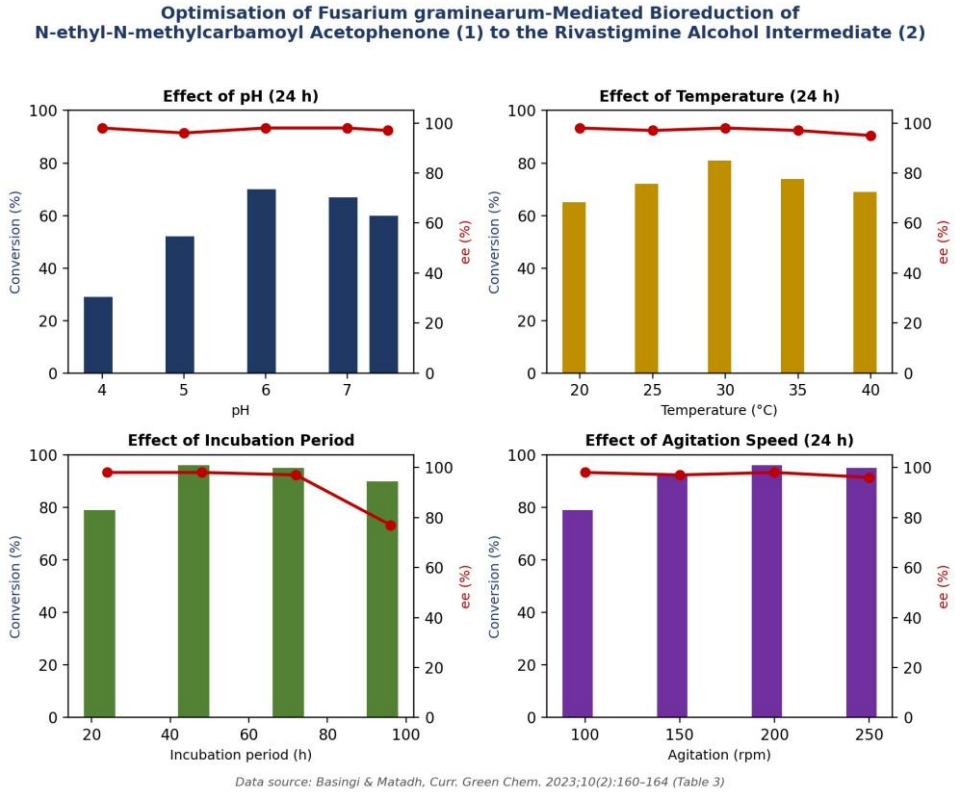


Figure 9. Effect of pH, temperature, incubation period and agitation speed on the conversion (bars, left axis) and enantiomeric excess (red line, right axis) of the Fusarium graminearum-mediated bioreduction of N-ethyl-

N-methylcarbamoyl acetophenone (1) to its chiral (S)-alcohol intermediate (2). Data reproduced from Basingi and Matadh, Current Green Chemistry, 2023;10(2):160–164 (Table 3).

Using these optimised fermentative conditions, gram-scale bioconversion was demonstrated at a substrate loading of up to 10 g L^{-1}, giving 96% conversion and 98% enantiomeric excess with only a slight decline in chiral purity (to 96% ee) observed at a higher loading of 15 g L^{-1}, attributed to possible substrate toxicity at elevated concentration. The overall process delivered the optically pure chiral alcohol intermediate in 82% isolated yield after silica-gel purification (Figure 10), starting from N-ethyl-N-methylcarbamoyl acetophenone that had itself been prepared by straightforward condensation of 3-hydroxyacetophenone with N-ethyl-Nmethylcarbamoyl chloride. This case study demonstrates that biocatalytic green chemistry for pharmaceutical intermediate synthesis is not confined to large, highly resourced pharmaceutical-industry/enzyme-engineering collaborations of the kind described for sitagliptin and atorvastatin (Sections 4.1–4.2): a newly isolated, uncharacterised environmental fungal strain, identified through a relatively modest soil-screening campaign and optimised using only standard fermentation parameters, was sufficient to deliver a synthetically useful, highly enantioselective route to a pharmaceutically relevant chiral building block.

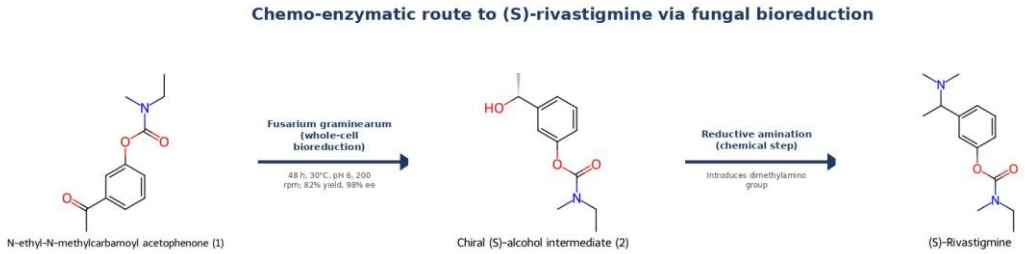


Figure 10. Chemo-enzymatic route to (S)-rivastigmine: whole-cell bioreduction of N-ethyl-N-methylcarbamoyl acetophenone (1) by the locally isolated fungus Fusarium graminearum gives the chiral (S)-alcohol intermediate (2) in 82% yield and 98% ee, which is subsequently converted to rivastigmine by a conventional reductive-amination step.

4.6 Other Notable Examples

Beyond the five case studies examined in detail above, the wider literature documents numerous further examples of biocatalysis displacing conventional chemical steps in API and intermediate manufacture. Deoxyribose-phosphate aldolase (DERA)-catalysed aldol cascades have been used for whole-cell production of lactonised statin side-chain building blocks common to several statins. Engineered ketoreductase platforms originally developed for atorvastatin have, as noted in Section 4.2, been successfully adapted for chiral-alcohol intermediates used in the synthesis of montelukast (an anti-asthma leukotriene-receptor antagonist) and duloxetine (an antidepressant). Enzymatic and two-step chemo-enzymatic routes to ursodeoxycholic acid, a bile-acid-derived hepatoprotective drug, using hydroxysteroid dehydrogenases (including a 7α-hydroxysteroid dehydrogenase from Ruminococcus torques) have similarly been developed as greener alternatives to the multistep chemical epimerisation historically used to invert the C7 stereocentre of chenodeoxycholic acid. Lipase- and esterase-catalysed kinetic resolutions continue to be used for a range of chiral alcohol and amine intermediates across multiple therapeutic classes, and rivastigmine itself has additionally been reported to be accessible via lipase- and ω-transaminase-catalysed chemoenzymatic routes distinct from the whole-cell fungal bioreduction described in Section 4.5. Collectively, these additional examples reinforce the pattern observed in the primary case studies: a naturally occurring enzyme or microbial isolate provides proof of chemical concept, and either directed protein evolution or systematic whole-cell process optimisation subsequently delivers the productivity and robustness required for commercial-scale, green pharmaceutical manufacture.

Table 1. Summary of the five principal case studies discussed in Section 4.

API / Intermediate	Biocatalyst / Organism	Reaction Catalysed	Reported Green Benefit
Sitagliptin	Engineered (R)-selective transaminase (Merck/Codexis)	Direct asymmetric amination of prositagliptin ketone	Eliminated high-pressure hydrogenation, rhodium catalyst and chiral purification; 19% less waste; 10–13% higher yield
Atorvastatin side chain	Ketoreductase + glucose dehydrogenase, then halohydrin dehalogenase (Codexis)	Asymmetric ketone reduction followed by chloro-to-cyano substitution	Replaced cryogenic, strongly basic Claisen chemistry with two mild, near-neutral aqueous steps; >99.5% e.e.

Pravastatin	Actinomycetes (Streptomyces carbophilus, Actinomadura sp., others)	Regioselective cytochrome P450-mediated 6hydroxylation of compactin	Avoids multistep, protectinggroup-dependent chemical hydroxylation of a complex polyketide substrate
Artemisinin (semisynthetic)	Engineered Saccharomyces cerevisiae expressing plant P450 (CYP71AV1)	Fermentative production of artemisinic acid from glucose via the mevalonate pathway	Decouples supply from a 14month agricultural crop cycle; stabilises price/availability for a global-health essential medicine
Rivastigmine intermediate	Locally isolated Fusarium graminearum (whole-cell fermentation)	Asymmetric bioreduction of an aryl ketone to a chiral secondary alcohol	82% yield, 98% ee at gram scale (10 g L^{-1}) using only standard fermentation optimisation, no enzyme engineering required

5. Complementary Green Technologies: Solvents, Flow Chemistry and Process Intensification

5.1 Green Solvent Selection

Because solvents typically constitute the single largest mass contributor to the E-factor of a pharmaceutical synthetic route, solvent selection has become a distinct sub-discipline of green chemistry in its own right. Beginning with Pfizer's Reagent Selection Guide in 2008, several major pharmaceutical manufacturers, including GSK, Sanofi and AstraZeneca, have published their own solvent selection guides that rank commonly used solvents by composite safety, health and environmental criteria, steering process chemists toward safer, more sustainable alternatives (such as ethanol, ethyl lactate and glycerol, several of which are derived from renewable feedstocks) and away from solvents of particular concern. These guides are frequently used alongside, rather than instead of, biocatalytic process redesign, since even an enzymatic or whole-cell fermentation step still requires a suitably green choice of extraction and work-up solvent — diethyl ether was used for product extraction in the rivastigmine intermediate case study (Section 4.5), for example — to realise its full sustainability benefit.

5.2 Continuous-Flow Biocatalysis

Continuous-flow processing, in which reagents are pumped through a reactor rather than processed in a single batch vessel, has increasingly been combined with biocatalysis to overcome several practical limitations of classical (batch) enzymatic catalysis, including timeconsuming work-up procedures, product- or substrate-mediated enzyme inhibition, and the difficulty of scaling up and intensifying batch bioprocesses. Flow biocatalysis has been applied to the synthesis of a range of APIs and natural products and is increasingly viewed as an important enabling technology for pharmaceutical companies seeking to remain both competitive and compliant with tightening regulatory and environmental expectations from agencies such as the U.S. Food and Drug Administration and the European Medicines Agency.

5.3 Energy-Efficient Activation: Microwave- and Ultrasound-Assisted Synthesis

Alongside biocatalysis, non-conventional energy-input methods such as microwave irradiation and ultrasound-assisted synthesis are increasingly used to accelerate pharmaceutical reactions, shorten reaction times, and reduce overall energy consumption relative to conventional thermal heating. Where these methods are combined with green solvents or solvent-free conditions, and with catalytic or biocatalytic steps, they contribute further to reducing the aggregate energy and mass intensity of a synthetic route, complementing rather than substituting for the specific selectivity benefits that biocatalysis provides for chiral pharmaceutical intermediates.

6. Comparative Environmental and Economic Impact of Bioconversion Routes

Across the five case studies reviewed in Section 4, a consistent pattern emerges: bioconversion routes tend to reduce waste and hazard not by eliminating any single “dirty” reagent in isolation, but by removing entire categories of process liability simultaneously — heavy-metal catalysts, cryogenic or high-pressure equipment requirements, strongly basic or acidic reagents, and dedicated chiral-resolution or purification steps (Figure 11). This compounding effect is part of why the reported improvements in the sitagliptin and atorvastatin case studies (double-digit percentage reductions in waste alongside simultaneous yield improvements) exceed what would typically be expected from a single-step process optimisation. Economically, these same simplifications frequently translate into lower capital equipment requirements (no need for high-pressure hydrogenation vessels, for example), lower rawmaterial and metal-catalyst costs, and reduced waste-treatment and disposal costs, which is why the pharmaceutical companies and enzyme-engineering firms involved in these case studies have generally continued to invest in and expand their biocatalysis platforms well beyond the original demonstration project. The rivastigmine intermediate case study (Section 4.5) illustrates a complementary, lower-cost route to the same category of benefit: rather than a multi-year, well-resourced directed-evolution campaign, a modest environmental soilscreening programme combined with standard fermentation-parameter optimisation was

sufficient to deliver a highly enantioselective (98% ee), reasonably high-yielding (82%) bioconversion, suggesting that meaningful green-chemistry gains are achievable at a range of resourcing levels, not only within large pharmaceutical/enzyme-engineering collaborations. At the same time, it is important to note that the sitagliptin and atorvastatin case studies represent some of the most successful and heavily resourced examples of biocatalytic process development in the industry; as discussed in Section 7, the underlying enzyme discovery and directed-evolution campaigns required to reach this point can themselves be lengthy and costly, and the net environmental and economic benefit of any given bioconversion route should therefore always be assessed on a case-by-case, whole-process basis rather than assumed a priori.

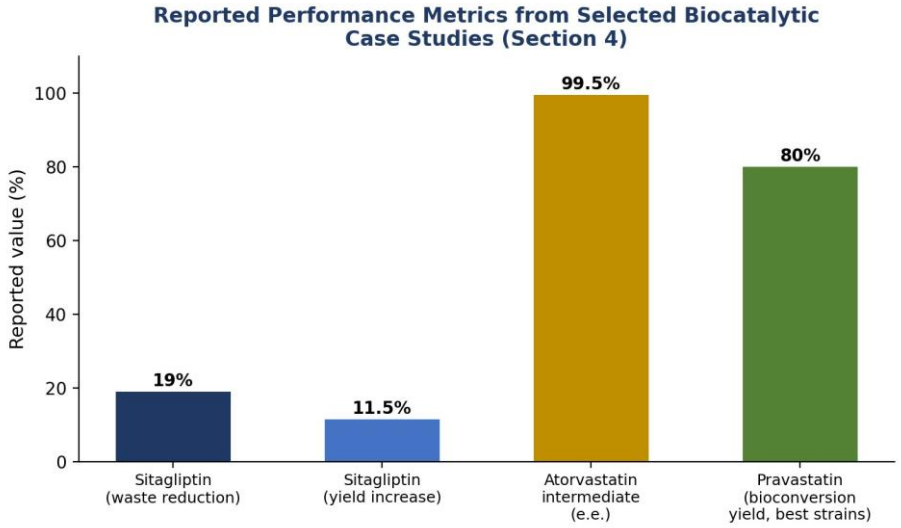


Figure 11. Selected reported performance metrics from the biocatalytic case studies discussed in Section 4: waste reduction and yield increase for the sitagliptin transaminase process; enantiomeric excess for the atorvastatin ketoreductase step; and best-reported whole-cell bioconversion yield among screened strains for the pravastatin-type hydroxylation.

7. Challenges and Limitations

Despite the substantial successes documented in Section 4, biocatalysis and bioconversion remain, by the admission of much of the reviewed literature, the exception rather than the norm in pharmaceutical organic synthesis, and several practical challenges continue to limit their wider adoption.

- Enzyme discovery and engineering cost and timeline: as illustrated by the sitagliptin transaminase (more than ten rounds of directed evolution, twenty-seven mutations) and the atorvastatin halohydrin dehalogenase (gene shuffling followed by further focused directed evolution), converting a marginally active naturally occurring enzyme into a commercially viable biocatalyst can require a substantial, multi-year research investment that is not guaranteed to succeed for any given target reaction.
- Limited substrate scope and enzyme/strain stability: many enzymes retain narrow native substrate specificity and can show reduced activity or stability under the higher substrate concentrations, non-aqueous cosolvents, and elevated temperatures often required for economically viable manufacturing-scale reactions; the rivastigmine intermediate case study (Section 4.5) similarly showed a decline in enantiomeric excess at extended incubation times (96 h) and at higher substrate loading (15 g L^{-1}), illustrating that even whole-cell fermentation systems have a practical operating window outside of which performance degrades.
- Cofactor dependence and regeneration: many of the most useful isolated-enzyme classes (ketoreductases, cytochrome P450 monooxygenases) require expensive cofactors such as NAD(P)H, necessitating an efficient, often multi-enzyme, cofactor-regeneration system that adds process complexity; whole-cell biocatalysts avoid this problem by regenerating cofactors through their own native metabolism, which is part of their continuing practical appeal for laboratories without access to isolated-enzyme engineering platforms.
- Multienzyme cascade compatibility: combining several enzymes in a single cascade (as in the two-step, three-enzyme atorvastatin process) requires that all catalytic components tolerate a common set of reaction conditions, which is not always readily achievable.
- Regulatory and quality considerations: introducing a biocatalytic step into an established, regulator-approved synthetic route requires demonstrating equivalent or superior impurity profiles and control strategies, which can represent a significant validation burden even where the underlying chemistry is well understood.
- Batch-to-batch biological variability: whole-cell microbial bioconversion processes, being biological fermentations, can be more susceptible to lot-to-lot variability in conversion efficiency than purely chemical unit operations, requiring robust strain identification, maintenance and process control of the kind described for the Fusarium graminearum isolate in Section 4.5.

These challenges explain why, notwithstanding the compelling environmental and economic case made by the landmark examples in this review, biocatalysis is generally adopted selectively — for specific, high-value, high-

impact synthetic steps where its selectivity advantage is decisive — rather than applied indiscriminately across an entire synthetic route.

8. Future Perspectives

Several converging developments are likely to shape the continued expansion of green chemistry and bioconversion methods in pharmaceutical API and intermediate synthesis. The integration of machine learning into enzyme engineering is already reducing the number of experimental rounds required to evolve a marginally active enzyme into an industrially viable biocatalyst, by computationally prioritising the mutations most likely to improve activity, stability or selectivity before they are made and tested in the laboratory. Systematic soil- and environment-based bioprospecting for novel whole-cell biocatalysts, of the kind described for the rivastigmine intermediate in Section 4.5, is likely to remain a valuable and comparatively low-cost complement to isolated-enzyme engineering, particularly for laboratories and research groups without access to directed-evolution infrastructure. Advances in genome mining continue to expand the pool of naturally occurring enzymes available as starting points for engineering campaigns, including newly characterised carbonyl reductases and cytochrome P450 systems relevant to statin and other API intermediate synthesis. Continuous-flow biocatalysis and other process-intensification technologies are expected to further improve the scalability and productivity of enzymatic and whole-cell bioconversion steps, addressing one of the recurring practical limitations identified in Section 7. Synthetic biology, exemplified by the semisynthetic artemisinin platform discussed in Section 4.4, offers a route to entirely reconstructing complex natural-product biosynthetic pathways in a well-characterised, fermentable host organism, a strategy that may extend to other structurally complex, currently plant- or fungus-derived APIs facing similar supply-security challenges. Finally, continued convergence and standardisation of green chemistry metrics — building on the ACS GCI Pharmaceutical Roundtable's work on process mass intensity — alongside their supplementation with life-cycle assessment and techno-economic analysis, will be important for enabling consistent, defensible comparisons between competing green and bioconversionbased synthetic routes as the field continues to mature.

9. CONCLUSION

Green chemistry and bioconversion methods have moved, over the past two decades, from promising academic concepts to proven, industrially and academically deployed technologies capable of delivering simultaneous environmental, safety and economic benefit in pharmaceutical API and intermediate manufacture. The five case studies examined in this review — sitagliptin, the atorvastatin side-chain intermediate, pravastatin, semisynthetic artemisinin, and a rivastigmine chiral intermediate produced by a locally isolated soil fungus — collectively demonstrate that biocatalysis and microbial bioconversion can eliminate hazardous reagents and energy-intensive process conditions, improve stereochemical purity, reduce waste generation, and in some cases address broader supply-security and global-health access concerns, across a spectrum of resourcing levels from major pharmaceutical/enzymeengineering collaborations to modest, single-laboratory soil-screening programmes. At the same time, the persisting technical, economic and regulatory challenges reviewed in this paper make clear that biocatalysis is best understood not as a universal replacement for conventional synthetic chemistry, but as a powerful, selectively applied tool that delivers its greatest benefit when directed at the specific synthetic steps — typically those requiring high stereoselectivity or difficult regioselective transformations — where its inherent advantages are most decisive. Continued investment in enzyme engineering, computational and machine-learning-guided biocatalyst design, environmental bioprospecting, continuous manufacturing technologies, and standardised green chemistry metrics will be central to extending these benefits across a wider range of the pharmaceutical industry's synthetic challenges in the years ahead.

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Conflict of Interest

The author(s) declare no conflict of interest.

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Data Availability

This is a review article; no new data were generated or analysed. Figures 8 and 9 reproduce/summarise previously published data as cited in the corresponding figure captions and reference list.

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