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Commercial Scale Biocatalysis: Myths and Realities

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Abstract—The unique ways in which enzymes are differentiated from other catalysts translate into special advantages. Understanding these advantages is the key toward better matching of biocatalysts needs in industrial chemistry. Specific cases where enzymes and biotransformations have been used successfully at the production scale are examined, permitting the realities of using biocatalysts to be separated from the misconceptions and myths. Five such misconceptions will be examined in the context of examples of some commercially-successful biocatalytic processes.

Introduction

The application of biocatalysis has received increased attention recently as the number of available enzymes has expanded rapidly. Natural diversity is being increasingly exploited to access enzymes with greater industrial utility.[1] High throughput screening techniques now permit the more rapid identification of new enzymes with desired characteristics. Mutation coupled with screening or selection methods has allowed the modification of existing enzyme activities, resulting in enzymes that are better suited to industrial needs.[2] These developments should make a significant impact on chemical process development in the future, particularly in the pharmaceutical and agricultural industries, where the products and intermediates are more amenable to biocatalytic synthesis.

However, preconceived ideas and misconceptions about enzymes continue to limit the commercial applications of enzymes and biotransformations in the chemical industry. Biocatalysis is still not considered a viable method by many synthetic chemists. Even those chemists who are familiar with enzymes often do not look to biocatalytic methods as a first-line alternative for the production of chemical intermediates. Too frequently, an enzymatic route is viewed as a course of last resort and attempted only after other possible synthetic schemes have failed. As a result, biocatalysis is not reaching its full potential.

Key words: Biocatalysis; enzyme; biotransformation; chiral intermediates.

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Most importantly, enzymes are catalysts. Enzymes are like other catalysts in that they simply catalyze the approach to equilibrium for a given reaction. However, enzymes are differentiated from other catalysts in that they are highly asymmetric, proteinaceous, and producible by fermentation from renewable resources.

The unique ways in which enzymes are differentiated from other catalysts translate into special advantages. Understanding these advantages is the key toward better matching of biocatalysts with needs in industrial chemistry.

The Facts

Perhaps the most important advantage that biocatalysts bring to organic synthesis is chemical precision. Such precision is most visibly demonstrated in terms of the remarkable chemical *selectivity* often displayed by enzymes. Better chemical selectivity leads directly to a number of commercial benefits, including better production of single stereoisomers, fewer side reactions, easier separation of products, and less pollution—all of which translate into lower cost.

Selectivity

The chemical selectivity of enzymes can be divided into four discrete categories:

- Substrate selectivity: the ability to distinguish and act on a subset of compounds within a larger group of chemically related compounds.
- Stereoselectivity: the ability to act on a single enantiomer or diastereomer selectively.

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- Regioselectivity: the ability to act on one location in a molecule selectively.
- Functional group selectivity: the ability to act on one functional group selectively in the presence of other equally reactive or more reactive functional groups.

Each type of chemical selectivity is described below. Biocatalytic reactions that exemplify each type of selectivity are used to show the benefits that can accrue to chemical processes that take advantage of the special properties of enzymatic catalysts.

Substrate selectivity. The substrate selectivity of enzymes for certain substrates is often amazing. Even when a crystal structure of an enzyme is available, the reason why one compound is bound and transformed while a similar compound is relatively inert defies easy explanation. Nonetheless, such selectivity can often be a powerful asset. For example, the broad-range transaminase from *Escherichia coli* can produce a fairly wide range of amino acids from the corresponding 2-ketoacids using either L-glutamic acid or L-aspartic acid as the amino group donor. L-Aspartic acid is particularly useful as the amino group donor according to Figure 1.[2]

The essentially irreversible decarboxylation of oxaloacetate to pyruvate drives the entire process to completion, with yields approaching 100% of theoretical.[3–6] The success of this reaction scheme rests on the fact that even though this enzyme accepts many different 2-ketoacids as substrates (2-ketoglutarate, 2-ketoadipate, phenylpyruvate, *p*-fluorophenylpyruvate, 4-thiomethyl-2-ketobutyrate, 3-imidazoylpyruvate, 3-*O*-benzylhydroxypyruvate, indoylpyruvate, and others), pyruvate is hardly acted on at all. Therefore, the reaction proceeds essentially to completion to produce the desired L-amino acid; the pyruvate co-product is unreactive, and as a result, little L-alanine is made, facilitating the recovery of the desired amino acid product.

Stereoselectivity. There are a number of examples of stereochemical specificity in enzyme catalysis at the commercial scale. The class of phenoxypropionic acid-based herbicides provides an example of a large volume agricultural product whose synthesis in stereochemically-pure form is based on enzymatic catalysis for the production of a key chiral intermediate. A dehalogenase enzyme from *Pseudomonas*, which acts selectively on the *R*-isomer of 2-halopropionic acids, is used to convert a racemic mixture of (*R,S*)-2-chloropropionic acid into an equimolar mixture of (*S*)-2-chloropropionic acid and (*S*)-lactic acid.[7] Reaction of (*S*)-2-chloropropionic acid with a substituted phenol provides the final product via a straightforward S_N2 displacement reaction. The overall reaction scheme is depicted in Figure 2.

Aspartame, the low-calorie sweetener and one of the most successful food ingredients ever launched, depends on biocatalysis to produce L-aspartic acid, one of its key components. The stereoselective addition of ammonia to fumarate catalyzed by the enzyme aspartase produces L-aspartic acid in high yield, high stereochemical purity, and low cost.[8,21] Processes in operation use either immobilized whole cells containing aspartase or isolated and immobilized aspartase enzyme. Currently, approximately 40,000 tons of L-aspartic acid are produced annually by this process by various producers around the world (Fig. 3).

Regioselectivity. Regioselectivity is another significant advantage of enzyme catalysis. Similarly reactive sites in a molecule can be differentiated by enzymes, as in the case of the enzymatic synthesis of aspartame. L-Aspartic acid contains two different carboxyl groups. Using thermolysin as a catalyst, only the alpha-carboxyl group of Z-aspartate reacts to form the amide with phenylalanine methyl ester; the beta-carboxyl group is unreactive. Aspartame is thus produced without contamination of the unwanted isomeric product (Fig. 4).[9]

Nowhere has the regioselectivity of enzyme catalysis been as exquisitely exploited as in the hydroxylation of steroids and their derivatives. Regioselective biocatalytic hydroxylations are known that display positional selectivity for almost every site on the steroid carbon skeleton. Since these positions are, for the most part, similar in chemical reactivity, such regioselectivity is virtually impossible to attain by any other means. It is also worth noting that these hydroxylation reactions are stereoselective as well (Fig. 5).

Functional group selectivity. Functional group selectivity can be extremely useful in cases where a reaction is desired on one chemical functional group in the presence of a chemically more reactive moiety. For example, the nitrilase-catalyzed hydrolysis of a nitrile in the presence of an ester or an amide is almost impossible to carry out using traditional chemical means, but through enzyme catalysis using a nitrilase, the reaction is straightforward and requires no special equipment or conditions.[17,18] Indeed, biocatalytic hydrolysis of a nitrile can be achieved without heat or the use of acid or base catalysis (Fig. 6).

Mild reaction conditions

The ability to catalyze reactions under mild conditions is another important benefit of enzymes as industrial catalysts. Enzymes typically function at ambient temperature, atmospheric pressure, and neutral pH. The generation of byproducts due to undesired side-reactions is minimized, raising yields and facilitating product recovery. One of the best examples of the advantage of mild reaction conditions is the production of acrylamide using a nitrile hydratase enzyme (Fig. 7).[10] Nitto Chemical has commercialized this process in Japan, where the scale of production is reported to be approximately 20,000 metric tons per year. Where the chemical process operates at temperatures of 80–140°C and always produces acrylic acid as a byproduct, the enzymatic process operates at 10°C and produces acrylamide in 100% yield with no acrylic acid byproduct. In addition, the chemical process uses a copper catalyst that generates toxic waste byproducts including HCN; such toxic

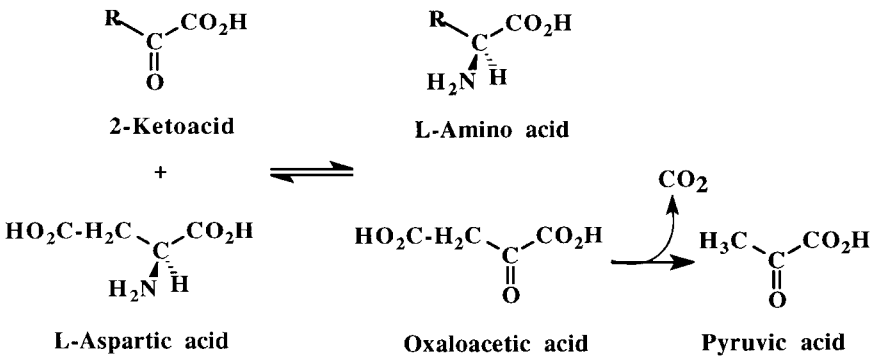


Figure 1. Production of amino acids by transamination.

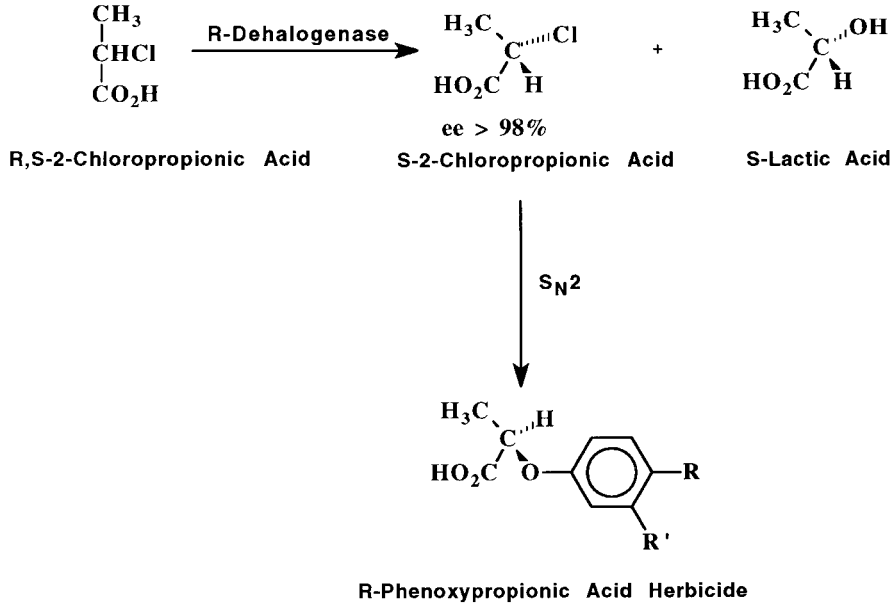


Figure 2. Biocatalytic step in the production of phenoxypropionic acid herbicides.

byproduct formation does not occur with the biocatalytic process.

Environmentally friendly

Enzymes are proteins, and as such are completely biodegradable. Biocatalysts composed of enzymes immobilized on inert materials such as silica or diatomaceous earth also pose no environmental hazards.

In addition to being biodegradable themselves, enzymes can result in processes that generate fewer waste disposal problems. When aqueous solutions are used, solvent consumption may be reduced.

Finally, the mild operating conditions of most enzymatic processes require lower energy input, leading to lower costs and lower emissions of greenhouse gases to the environment.

High catalytic efficiency

Enzyme-catalyzed reactions usually display characteristically high turn-over numbers, with rate accelerations approaching or exceeding 10^8. Considering that catalysis is normally carried out at ambient temperature and pressure, the catalytic power of enzymes is all the more remarkable. The chemoselective hydroxylation of steroids is an example of the multiple benefits that can accrue from biocatalyzed reactions. Hydroxylation of an unactivated C–H bond is difficult chemically, requiring aggressive conditions to carry out. Selectivity in a complex hydrocarbon is virtually impossible to achieve. Yet, as described earlier, steroid hydroxylases carry out this reaction near ambient temperature, and with high regioselectivity and essentially perfect stereoselectivity.

The catalytic potential of enzymes can also be seen in the industrial process for L-aspartic acid. Aspartate ammonia lyase is used as the catalyst, either by immobilizing whole cells containing the enzyme or by immobilization of the isolated enzyme itself. The immobilized enzyme is stable and highly active. In this process, a single kilogram of immobilized enzyme produces 10,000 to more than 100,000 kilograms of product, making it one of the most efficient biocatalytic processes known. Such efficiency is reflected in the price for L-aspartic acid, which is about $3.00 per kilogram.

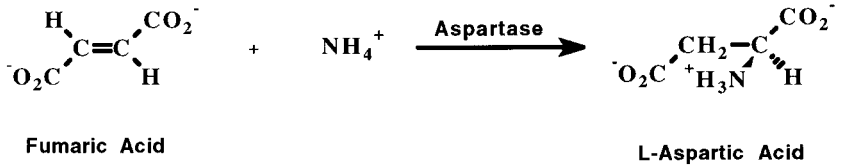


Figure 3. Production of L-aspartic acid.

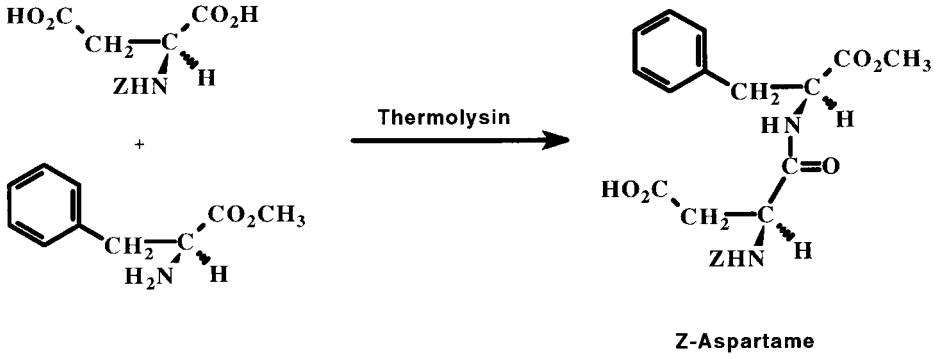


Figure 4. Biocatalytic production of aspartame.

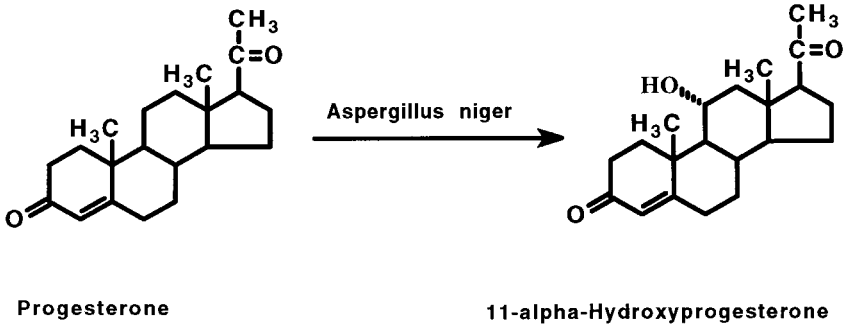


Figure 5. Regioselective hydroxylation of progesterone.

The Myths

In spite of the advantages of biocatalysis, it is still relatively underutilized as a technology. Biocatalysis is still not considered a viable method by many synthetic chemists. Even those chemists who are familiar with enzymes often do not look to biocatalytic methods as a first-line alternative for the production of chemical intermediates. Too frequently, an enzymatic route is viewed as a course of last resort and attempted only after other possible synthetic schemes have failed. Preconceived ideas and misconceptions continue to limit the commercial applications of enzymes and biotransformations in the chemical industry. As a result, biocatalysis is not reaching its full potential.

There are a number of long-standing beliefs about what biological catalysts can and cannot do. Many of these beliefs stem from things "learned" in the past—for example, that enzymes require aqueous conditions to function. Today there are multiple examples that demonstrate the ability of enzymes to function in non-aqueous milieu, effectively converting this myth into a reality. In this case and in many others as well, knowledge is the best agent of change.

As chemists gain knowledge of enzymes and a better appreciation of the capabilities of biocatalysts, perceptions will change. Let us examine 5 commonly-held ideas about enzymes. Our goal is to assess their accuracy based on recent developments and discoveries, and to the extent they prove to be misconceptions (myths), an opportunity arises.

Myth No. 1: Enzymes are too expensive

At first glance, this statement would appear to be almost irrefutable. Enzymes are, generally-speaking, expensive—at least when priced on a cost per mol or even a cost per unit weight basis.

As shown in Table 1, in bulk, prices of most enzymes range from a high of $100,000 per kilogram for a diagnostic enzyme such as lactic dehydrogenase to $100 per kilogram for bulk, crude preparations of amylases. For most enzymes we consider for biocatalytic applications, such as penicillin amidase, thermolysin, aspartase, or a transaminase, prices generally fall in the range of $500 to $20,000 per kilogram, depending on purity. However, the key cost to consider in biocatalysis is not the cost of the enzyme itself, but rather, the cost-contribution of

Table 1. Bulk enzyme prices

Enzyme	Approx. price in $/kg
Lactic dehydrogenase	100,000
Porcine liver esterase	15,000
Penicillin amidase	10,000
Aspartase	10,000
Trypsin	5000
Lipase	5000
Glucose isomerase	500
Detergent protease	250
Glucoamylase	100

Table 2. Bulk catalyst prices

Catalyst	Approx. price in $/kg
BINAP	40,000
ChiraPhos	10,000
Platinum	12,000
Sharpless	10,000
$Pd(Diphos)_2$	5000
$Rh(PPh_3)_3Cl$	2000
Jacobsen	1000
Chirald	500
Raney nickel	30

the enzyme to the final product. Even at the prices shown in Table 1, that cost contribution can be reasonable, and even surprisingly low. For example, the cost contribution of a transaminase for the production of *p*-fluoro-L-phenylalanine, which may have a selling price of $500 per kilogram or more, is only about $20–30 per kilogram. This cost contribution is far less than that of the raw materials. The cost contribution for penicillin amidase in the splitting of penicillin G is only about $1 per kilogram. And the cost contribution of aspartase in the production of L-aspartic acid is less than $0.10 per kilogram!

Finally, as shown in Table 2, when compared with the cost of other catalysts—especially those that are similarly selective—the prices of enzymes are not very different.

Myth No. 2: Enzymes are too unstable

The question is "Unstable to what?" Many enzymes are unstable toward high temperatures or extremes of pH. Solutions of enzymes sometimes lose activity on standing at room temperature. However, the important aspect of stability is the stability under operational conditions for a desired process.

There are many examples of enzymes that display excellent stability when used in a process setting, especially when immobilized. Aspartase, lipases, amidases, hydantoinases are just some of the types of enzymes that have shown half-lives of weeks or months under process conditions. Table 3 lists a number of examples of biocatalytic processes using immobilized enzymes that have shown sufficient stability for scale-up and commercialization.

Furthermore, the development of thermostable enzymes by isolation from thermophiles or by directed evolution promises to create even more robust catalysts for industrial application. Stability, while always a concern for any catalyst, is becoming less of a concern for enzymes as scientific progress addresses the stabilization of enzymes under a range of conditions.

Myth No. 3: Productivity is too low

> "Microbial processes are always considerably slower than traditional chemical processes"
>
> Source: *Biocatalysts in Organic Synthesis*, 1985.

It is often assumed that enzymes and biocatalytic processes are inherently low in volumetric productivity, and many quotes to this effect can be found in the literature. This belief probably has arisen due to parallels drawn from fermentation processes, where volumetric productivities are almost always less than 1 g/L/h. However, a fermentation process typically involves biosynthesis of

Figure 6. Chemo-selective hydrolysis of a nitrile.

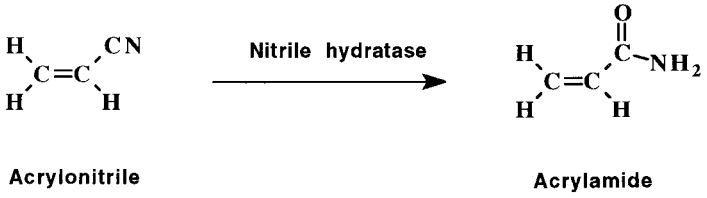


Figure 7. Biocatalytic production of acrylamide.

Table 3. Reported operational half-lives for immobilized biocatalysts

Biocatalyst	Reported half-life	Source
Aspartase	6 months–2 years	Sato et al.,[23] Rozzell et al.[3–6]
Isomaltulose synthase	358 days	Cheetham et al.[22]
Urocanase	180 days	Chibata et al.[8]
Fumarase	180 days	Takata et al.[24]
Arginine deiminase	140 days	Chibata et al.[8]
Transaminase	90 days	Rozzell et al.[3–6]
Penicillin amidase	> 6 months	Röhm Pharma
Lactase	90 days	Messing et al.,[25] Goldberg[28]
Protease	> 60 days	Dierenfeldt and Rozzell[26]
Glucose isomerase (60°C)	> 60 days	Novo Industri
Tryptophan synthase	50 days	Wagner et al.[27]
Cyclodextrin glu. trans.	> 45 days	Crump and Rozzell[2]
Asp.-ß-decarboxylase	45 days	Rozzell et al.[3–6]
Hydantoinase	30 days	Syldakt et al.[29]

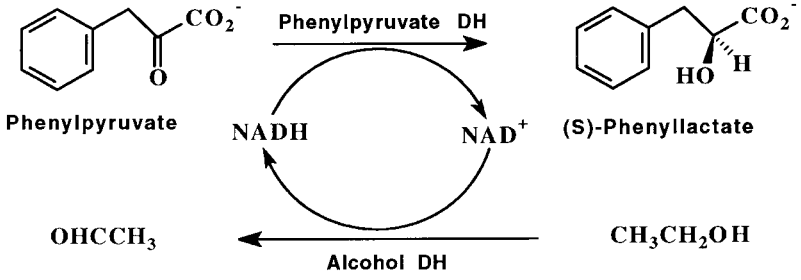


Figure 8. Cofactor recycling using a coupled enzyme reaction.

a metabolite from sugars and other fundamental raw materials through an entire pathway from carbohydrate precursors.

If we consider single step enzymatic transformations, the situation is simpler, and the result can be quite different. Productivities of tens or even hundreds of grams of product per liter per hour have been achieved using immobilized enzyme processes.

Examples of highly productive biocatalytic processes include the production of L-aspartic acid by immobilized aspartase, the production of L-phenylalanine using an amidase or a transaminase, the production of L-*tert*-leucine using leucine dehydrogenase in a membrane reactor, the production of 2-chloropropionic acid using lipase-catalyzed esterification, and the production of 1-phenylethylamine by stereoselective lipase-catalyzed acylation.

Myth no. 4: Redox cofactors cannot be recycled efficiently

The notion that redox cofactors (and this most commonly means nicotinamide cofactors) cannot be recycled efficiently has led to suggestions in the past that one the characteristics of a successful biocatalytic process is that is does not require cofactors. While it is true that nicotinamide cofactors are not always easy to recycle, there is a building body of evidence that such cofactors can be recycled economically in many instances. In fact, there are three ways that have been demonstrated at the industrial scale for the recycle of cofactors. Each will be examined below in some detail.

First method: use a coupled reaction. As an example, phenyllactic acid is produced by the stereoselective reduction of phenylpyruvate using NADH and a phenyllactate dehydrogenase (Fig. 8). The NADH required for reduction is regenerated using alcohol dehydrogenase and ethanol.[19] The recycle number achieved is approximately 2,000, and ethanol is an inexpensive reductant, resulting in an economic process. Formate dehydrogenase has also been used in a similar fashion for regeneration of NADH, using formate as the reductant.

Second method: use a macro-molecularized cofactor in a membrane reactor. Wandrey, Kula, and co-workers in cooperation with Degussa AG have demonstrated the use of a macro-molecularized cofactor retained in a membrane reactor as an efficient method for recycling nicotimamide cofactors.[11] NAD^+ is chemically attached to polyethylene glycol of molecular weight approximately 10,000–20,000, increasing the effective molecular weight of the cofactor. This permits the cofactor to be retained inside a membrane reactor with the enzyme, while small molecules such as substrates and products pass through the membrane freely. Currently, the non-naturally occurring amino acid L-*tert*-leucine is manufactured using the process scheme shown in Figure 9.

Recycle numbers as high as 600,000 have been reported for this type of reaction system, rendering the cost of the cofactor an insignificant part of the overall cost of production.[16]

Third method: use whole cells and a carbon source. The third method of efficient cofactor recycling involves the use of whole cells containing both the dehydrogenase enzyme and the cofactor.[20] Recycling of the cofactor is achieved through the addition of a carbon source that provides both maintenance energy for the cell and reducing equivalents for cofactor regeneration.

An example of the use of this method is the production of the chiral alcohol in Figure 10 as an intermediate for the synthesis of benzodiazepine drug under development at Eli Lilly and Company.

A food-grade yeast, *Z. rouxii*, was found that could reduce the ketone precursor with high enantioselectivity, producing the (*S*)-isomer in greater than 99% enantiomeric excess. By the addition of 2 kg of glucose per kilogram of product to the reaction medium, cofactor regeneration occurred smoothly inside the cells, providing an inexpensive method for biocatalytic reduction.[12]

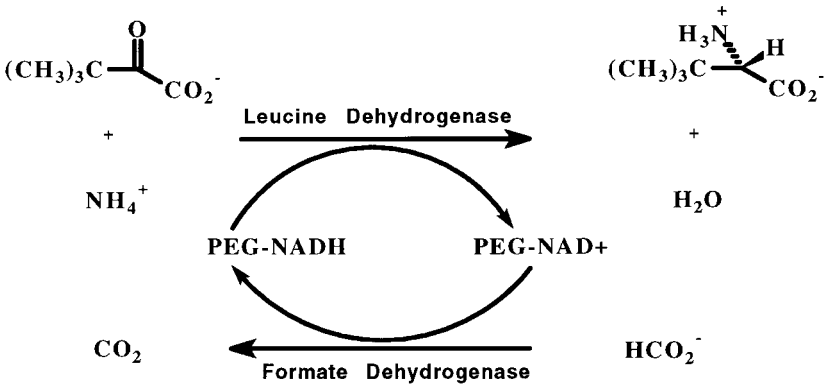


Figure 9. Cofactor recycling in the production of L-*tert*-leucine.

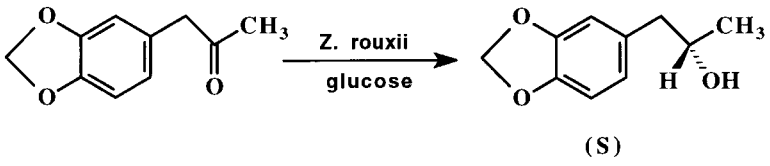


Figure 10. Cofactor recycling using whole cells.

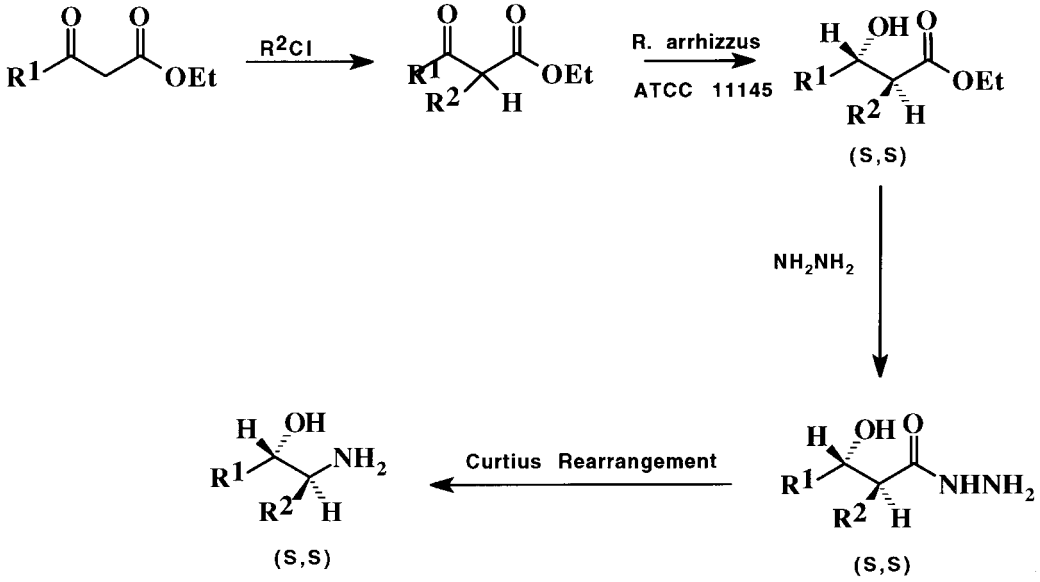
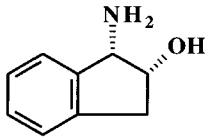
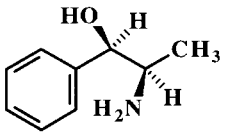


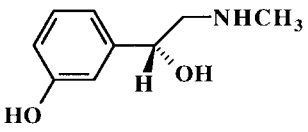
Figure 11. Method for the production of chiral vicinal aminoalcohols.



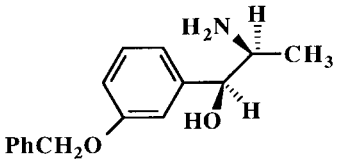
(1S,2R)-1-Amino-2-Indanol



(1R,2R)-1-Hydroxy-2-Amino-1-Phenylpropane
Precursor for Pseudoephedrine

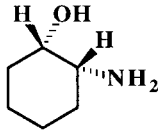


Phenylephrine

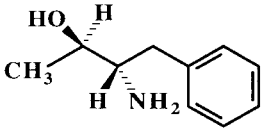


Metaraminol

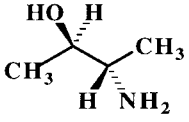
Thiamphenicol



(1R,2S)-1-Amino-2-Hydroxycyclohexane



(2S,3R)-2-Amino-3-Hydroxy-1-Phenylbutane



(2S,3R)-2-Amino-3-Hydroxybutane

Figure 12. Examples of commercially-important chiral vicinal aminoalcohols.

A number of other reductions using whole microbial cells have been described at the laboratory scale.[13] Given the research effort in this area, there is a strong likelihood that similar processes will appear in the near future. One such application under development uses a stereoselective reduction of a 2-substituted-ß-ketoester. Because carbon-2 is adjacent to both the ester and keto groups, it readily enolizes prior to reduction, maintaining rapid equilibrium between the enantiomeric forms of the ß-ketoester. Stereoselective reduction occurs on only one of these enantiomeric ß-ketoesters, producing a 2-substituted-ß-hydroxyester with a high degree of control over the absolute configuration at two chiral centers simultaneously. By choosing the appropriate organism for the stereoselective reduction, a single diastereomer of the four possible stereoisomers can be generated in high yield and stereochemical purity. The resulting chiral 2-substituted-ß-hydroxyester is transformed chemically to a corresponding chiral vicinal aminoalcohol as shown in Figure 11.[14]

Vicinal aminoalcohols are important compounds as pharmaceutically-active substances. They have further applications as intermediates for a number of important pharmaceutical products, including pseudoephedrine, epinephrine, norepinephrine, metaraminol, and the HIV-RT inhibitor indinavir (Crixivan).[15] Some of these commercially-important chiral vicinal aminoalcohols are shown in Figure 12.

Table 4. Products manufactured using biocatalytic reactions

Product	Approx. world market ($MM)
Fatty acids/triglyceride oils	1000
High fructose corn syrup	1000
Aspartame	800
Acrylamide	300
6-APA/7-ADCA	200
Amino acids	150
S-2-Chloropropionic acid	10
Cyclodextrins	10

Myth No. 5: Enzymes don't catalyze industrially interesting reactions

A common reason given not to use enzyme-catalyzed reactions is that they do not work on reactions of industrial interest. The tendency is to assume that compounds of non-biological origin will not be well-accepted or well-tolerated by the enzyme, or that typical synthetic organic reactions are not amenable to enzymatic catalysis. Yet, there is a biocatalytic counterpart for almost every chemical reaction known.

Enzymes have limitations, and are unlikely to ever be useful in cracking hydrocarbons. However, within the limitations of biocatalysis, however, is a vast range of capabilities. Enzyme can function in either aqueous or non-aqueous solvent systems, and can act on virtually any kind of chemical starting material. Table 4 lists a few of the biocatalytic reactions that have been commercialized successfully, displaying the scope of biocatalysis to date.

The classes of reactions catalyzed, and the types of products made by biocatalysis will only expand over time as increased activity in the application of biocatalysis delivers new processes for chemical synthesis.

The Realities

As with any broad generalizations, there are almost always exceptions. However, the preceding examples show that much of what was thought to be true of enzymes has been shown not necessarily to be fact, and certainly not to be broadly applicable. In fact, there are industrially-successful examples of biocatalytic processes that show enzymes to be sufficiently stable, productive, and economic for commercial application. Enzymes have enormous breadth of scope in the types of reactions that may be catalyzed and the types of compounds that may be acted on. Enzymes can function under industrially-useful conditions, including in the presence of organic solvents. And relatively complex enzyme systems, such as those that use redox cofactors, can be scaled-up and commercialized.

Biocatalysis is clearly no panacea. As with any emerging technology, ''start-up'' difficulties in ''proceeding up the learning curve'' will be encountered. However, it is clear that biocatalysis is a useful weapon to have in the chemical arsenal. Certain applications are better-suited for enzymes and biotransformations than others. By examining long-held beliefs about enzymes, one can separate fact from myth, leading to a better understanding of what biocatalysts can do well, and conversely, what they do poorly. This knowledge will enable better selection of targets for biocatalysis in the future, while helping to avoid significant investment in processes for which biocatalysts are ill-suited and less likely to succeed.

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