classified solvents into three categories: preferred, usable, and undesirable with an advice regarding substitution of undesirable solvents [16, 38]. Sanofi scientists [39] divided solvents into four categories based on safety, health, and environmental hazards and other industrial issues: (i) recommended, (ii) substitution advisable, (iii) substitution requested, and (iv) banned. Similarly, GSK has a similar guide, with two safety criteria, one health criterion, three environmental criteria including life cycle scoring, and additional red flags, for example, for solvents governed by regulations [40, 41]. Solvents derived from renewable feedstocks, such as ethanol, ethyl lactate, and methyl tetrahydrofuran [42], are becoming popular reaction media as they are seen as "natural" and sustainable.

In the original inventory of E factors of various processes, we assumed [3], if data were not available, that solvents would be recycled by distillation and that this would involve a 10% loss. However, this was probably overoptimistic, certainly for the pharma industry where the widespread use of different solvents for the various steps in multistep syntheses makes recycling difficult owing to cross contamination. The best solvent is no solvent, but if a solvent is needed, it should be safe to use and there should be provisions for its efficient removal from the product and reuse.

1.5 THE ROLE OF CATALYSIS

The waste generated in the manufacture of fine chemicals and pharmaceuticals is largely due to the use of stoichiometric inorganic and organic reagents that are partially incorporated or not incorporated into the product. Typical examples include oxidations with inorganic oxidants such as chromium (VI) salts, permanganates, manganese dioxide, and stoichiometric reductions with metals (Na, Mg, Zn, Fe) and metal hydrides ($LiAlH_4$, $NaBH_4$). Similarly, stoichiometric amounts of mineral acids (H_2SO_4, HF, and H_3PO_4) and Lewis acids ($AlCl_3$, $ZnCl_2$, BF_3) are major sources of waste. The solution is evident: the substitution of antiquated stoichiometric methodologies with cleaner catalytic alternatives [43–45]. This is true elegance and efficiency in organic synthesis [46]. For example, catalytic hydrogenation, oxidation, and carbonylation are highly atom-efficient processes. Similarly, the use of recyclable solid (heterogeneous) acids and bases as catalysts results in substantial reductions in waste in industrial organic synthesis [47, 48]. Indeed, several pharma companies have developed reagent guides for particular reaction types with the aim of improving the greenness and sustainability of their processes [41].

The ultimate in step and AE is the development of catalytic cascade processes whereby several catalytic steps are integrated in one-pot procedures without the need for isolation of intermediates [49]. Such "telescoping" of multistep syntheses into catalytic cascades has several advantages—fewer unit operations, less solvent and reactor volume, shorter cycle times, higher volumetric and space-time yields, and less waste (lower E factor)—that afford substantial economic and environmental benefits. Furthermore, coupling of reactions can be used to drive equilibria toward product, thus avoiding the need for excess reagents.

1.6 BIOCATALYSIS AND GREEN CHEMISTRY

Biocatalysis has many attractive features in the context of green chemistry and sustainable development:

1. The catalyst (an enzyme) is derived from renewable resources and is biocompatible (sometimes even edible), biodegradable, and essentially nonhazardous, that is, it fulfills the criteria of sustainability remarkably well.

2. Biocatalysis avoids the use of, and contamination of products by, scarce precious metals such as palladium, platinum, and rhodium. The long-term commercial viability of many "endangered" elements, such as various noble metals, is questionable. Moreover, the costs of removing traces of noble metals, to an acceptable level, from end products can be substantial.
3. Reactions are performed in an environmentally compatible solvent (water) under mild conditions (physiological pH and ambient temperature and pressure).
4. Reactions of multifunctional molecules proceed with high activities and chemo-, regio-, and stereoselectivities and generally without the need for functional group activation, protection, and deprotection steps required in traditional organic syntheses. This affords processes that are more step economic and more efficient in energy and raw material consumption, generate less waste, and are, therefore, both environmentally and economically more attractive than conventional routes.
5. As a direct result of the higher selectivities and milder reaction conditions, biocatalytic processes often afford products in higher purity than traditional chemical or chemo-catalytic processes.
6. Enzymatic processes (but not fermentations) can be conducted in standard multipurpose batch reactors and, hence, do not require any extra investment, for example, for high-pressure equipment.
7. Biocatalytic reactions are conducted under roughly the same conditions of temperature and pressure, and, hence, it is relatively easy to integrate multiple reactions into eco-efficient catalytic cascade processes [50].

In short, biocatalysis fits very well with the principles of green chemistry and sustainability. As Barry Commoner, the doyen of industrial ecology, observed [51]: "in nature there is no such thing as waste, everything is recycled." As shown in Table 1.2, biocatalysis conforms with 10 of the 12 principles of green chemistry and is not really relevant for the other two (principles 4 and 10), which are concerned with the design of safer, biodegradable products. Consequently, since the mid-1990's, biocatalysis has emerged as an important technology for meeting the growing demand for green and sustainable chemical manufacture [52, 53], particularly in the pharmaceutical industry [54, 55].

Thanks to advances in biotechnology and protein engineering techniques such as *in vitro* evolution [56], it is now possible to produce most enzymes for commercially

TABLE 1.2 Biocatalysis and the Principles of Green Chemistry

Green Chemistry Principles	Biocatalysis
1. Waste prevention	Enables more sustainable routes with significantly reduced waste
2. Atom economy	Enables more atom and step economic routes
3. Less hazardous syntheses	Generally low toxicity
4. Design for safer products	Not relevant
5. Safer solvents and auxiliaries	Usually performed in water or Class 3 solvents
6. Energy efficient	Mild conditions are conducive with energy efficiency
7. Renewable feedstocks	Enzymes are renewable
8. Reduce derivatization	Biocatalysis obviates the need for protection/deprotection
9. Catalysis	Enzymes are catalysts
10. Design for degradation	Not really relevant but enzymes themselves are biodegradable
11. Real-time analysis for pollution prevention	Can be applicable in biocatalytic processes
12. Inherently safer processes	Performed under mild and safe conditions

acceptable prices and to manipulate them such that they exhibit the desired properties with regard to, *inter alia*, substrate specificity, activity, selectivity, stability, and pH optimum [57, 58]. This has made it eminently feasible to optimize the enzyme to fit a predefined optimum process that is genuinely benign by design. Furthermore, the development of effective immobilization techniques has paved the way for optimizing the storage and operational stability and the recovery and recycling of enzymes [59]. In addition, the coimmobilization of two or more enzymes can afford multifunctional solid biocatalysts capable of catalyzing biocatalytic cascade processes [60].

Biocatalytic processes are performed with isolated enzymes or as whole-cell biotransformations. Isolated enzymes have the advantage of not being contaminated with other enzymes present in the cell. The use of whole cells, on the other hand, is less expensive as it avoids the separation and purification of the enzyme. In the case of dead cells, E factors of the two methods are essentially the same: the waste cell debris is separated before or after the biotransformation, respectively. In contrast, substantial amounts of waste biomass can be generated when using growing microbial cells in the fermentation processes. We note, however, that this waste is generally easy to dispose of, for example, as animal feed or can, in principle, be used as a source of energy for the process. Many fermentation processes also involve the formation of copious amounts of inorganic salts that may even be the major contributor to waste. E factors have generally not been calculated for fermentations, but published data [61] regarding mass balances can be used to calculate E factors. The E factor for the bulk fermentation product—citric acid, for example—is 1.4, which compares well with the E factor range of <1–5 typical of bulk petrochemicals. Interestingly, ca. 75% of the waste is accounted for by an inorganic salt, calcium sulfate. If water is included in the calculation, the E factor becomes 17. In contrast, small-volume fermentation processes for low-volume, high-added-value biopharmaceuticals can have extremely high E factors, even when compared with those observed in the production of small-molecule drugs. The fermentative production of recombinant human insulin [15], for example, involves an E factor of ca. 6600 and inclusion of water affords an astronomical E factor of 50 000! In contrast, biocatalysis with isolated enzymes tends to involve significantly higher substrate concentrations and combines a higher productivity with a lower water usage compared to fermentations.

1.7 EXAMPLES OF GREEN BIOCATALYTIC PROCESSES

1.7.1 A Chemoenzymatic Process for Pregabalin

Pfizer scientists have described [62] a second-generation chemoenzymatic process (Figure 1.3) for the manufacture of pregabalin, the active ingredient of the CNS drug Lyrica. It represented a dramatic improvement in process efficiency compared to earlier routes. The stereocenter was set early in the synthesis in accordance with the golden rule of chirotechnology [63], and the wrong enantiomer could be easily racemized and reused. The key enzymatic step was conducted with an inexpensive, readily available laundry detergent lipase at a staggering substrate concentration of 765 g/l. Organic solvent usage was dramatically reduced in a largely aqueous process. Compared to the first-generation manufacturing process, the new process afforded a higher yield and a fivefold reduction in the E factor from 86 to 17.

1.7.2 A Three-Enzyme Process for Atorvastatin Intermediate

Codexis scientists developed and commercialized a green-by-design, three-enzyme process for the synthesis of a key intermediate (Figure 1.4) in the manufacture of atorvastatin, the active ingredient of the cholesterol-lowering drug Lipitor [64, 65]. In the first step, ethyl-4-chloroacetoacetate undergoes highly enantioselective reduction