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GENERAL BIOGRAPHY, STRUCTURE AND CLASSIFICATION OF ENZYMES

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Abstract

Enzymes are a group of biocatalysts with protein structure produced from bacteria, yeasts, fungi, plants, and molds, which have received attention in recent years due to their region-, stereo-, and enantioselectivity. Because of these features, enzymes are used in a wide range of organic synthesis interestingly especially in asymmetric synthesis and kinetic resolution to obtain pure enantiomers. All groups of enzymes are produced from renewable raw materials and are biodegradable and exhibit different properties, including stability, activity, and specificity. Biocatalysts present many appealing features in green chemistry and because of the mild operating conditions of enzymatic processes, they can be performed in relatively simple equipment and are easy to control. In the current review, we discuss on the biography and structure of enzymes in general. In addition, the classification of enzymes is reviewed.

1. Introduction

Enzymes have protein structure and consider as potential chiral catalysts for a wide range of processes. In generally, biocatalysts are applied in the transformation of macrostructures to new materials and energy, besides for growth, maintenance, and repair of cells [1]. Many chemical reactions occur by living cells where extraordinary complex systems are there. Enzymes are biocatalysts that cause many chemical reactions happen. In the absence of enzymes, the chemistry occurred by living cells would happen at too slow rate for the organism to stay alive and called metabolism. The history of enzymes and applied biocatalysts goes back to ancient Egypt from thousands of years where enzymes were obtained from microorganisms and used in alcohol production, cheese making, cooking, baking, and brewing. This has been exhibited in an old Egyptian papyrus showing approaches used to preserve alcoholic drinks and food (Figure 1). The same applications were used in ancient Greece [2, 3].

Figure 1. Enzyme-catalyzed beer manufacturing in ancient Egypt [2].

The structure of enzymes includes a protein with a primary structure of covalently linked amino acids that obtain the sequence sheet folds, helical, and globular based on significant hydrogen bonding (i.e., secondary structure). The interactions of secondary structures form a tertiary structure. These interactions also form the basis of the catalytic cleft of the protein for activity of enzymes: satisfying requirements with the solvent, maximizing hydrophobic interactions, and forming salt bridges. Since exposure of these residues (e.g., phenylalanine, tyrosine, etc.) to a native hydrophilic environment would be unfavourable, a core of hydrophobic amino acids is given to enzymes by this tertiary structure. The tertiary structures also tend to yield basic or acidic amino acids on the surface due to their activity to hydrogen bond [4].

Using enzymes for asymmetric synthesis and kinetic resolution to obtain pure enantiomers has been increased dramatically [5]. During the past decades, enzymes have been used as chiral catalysts within the tool kit of the organic chemist [6]. Enzymes are biocatalysts that have many properties like high selectivity, activity, and specificity that allow performing the most complex organic reactions under the non hazardous environmental and experimental conditions [7]. Enzymes present many attractive properties in the field of green chemistry, especially for production of chiral building blocks to synthesis the enantiopure drugs or food products: mild reaction conditions (physiological temperature and pH, aqueous medium as the common solvent, although many other green solvents can be applied) and an ecofriendly catalyst (a cell or an enzyme) displaying high activities and regio-, and chemo-, stereo selectivities in

complex molecules [8]. Recently, scientists have used enzymes for performing regio-, chemo-, enantio-, diastereo, and prochiral selective acylation/deacylation reactions on a variety of substrates, such as alcohols, polyphenolics, acids, sugars, nucleosides, etc. [9]. Enzymes are no exception, can be active efficiently under very mild conditions. They must perform well at mild conditions and compatible with the preservation of cell viability. They are able to increase reaction rates by $10^6 - 10^{12}$ and are limited only by how fast the next substrate enters the active site. Enzymes are used in many fields, including organic synthesis, pharmaceuticals, fine chemicals, clinical analysis, fermentation, food production, and detergents [10-13].

Biocatalysts can chose and catalyze a specific part of molecules, produce pure yield, and repeat the reaction many times over without any error by using conditions such as atmospheric pressure, near neutral pH in aqueous systems, and temperatures under 37°C [14].

There is one or more of the following problems should have to be considered using enzymes:

(i) Biocatalysts activity and stability: A major bug of enzymatic processes is their low stability and/ or activity under working conditions, and various other disadvantages may occur and minimize enzyme usability, like product- and substrate-inhibitions, which reduces the economic use of the respective enzyme.

(ii) Substrate range: Enzymes are commonly modified for defined natural substrates. As a result, non-natural reactants are usually changed with notably lower activity, if converted at all. However, we can overcome to these limitations by using enzyme and reaction engineering.

(iii) Non-enzymatic reaction: Unfortunately, there is numerous undesirable side reactions such as decomposition reactions of substrates and products can be occur beside the desired reaction [15]. In this review, we focus on the biography, structure, and classification of enzymes.

2. Enzyme Biography

Enzymes are nature's catalysts which convened from primary amino acid trails by nature to generate 3D structures with precise geometries. Biocatalysts can catalyze transformations with precious fidelity and selectivity in aqueous media under noble conditions [16, 17].

Enzymes exist in filamentous fungi, yeast, and bacteria. Firstly, enzymes were isolated and applied in the year 1914, their protein nature proved in 1926 and in 1960s the large-scale microbial production of enzymes was started. The industrial enzyme business is significantly thrived because of engineered enzyme features, improved synthesizing sciences, and novel application fields. A wide range of useful enzymes with differences in substrate specificity, reaction rate, thermal stability, and optimal pH are produced by native or recombinant micro-organisms. Fermentation processes facilitate obtaining microbial enzymes. One of the most important factors in enzyme-catalyzed reactions is the choice of enzyme sources. Enzymes obtained from various sources always exhibit different characteristics, such as stability, activity, specificity, and so on. Commercial suppliers offer several enzymes from different sources and few vendors also offer enzyme-screening sets are also offered by few vendors (Table 1) [18, 19]. The selection of enzyme sources is one of the most important parameters for enzyme-catalyzed reactions. Enzymes derived from a variety of micro-organisms as source including bacteria, yeasts, fungi, plants, and molds exhibit different properties, including stability, activity, and specificity [20-27].

Table 1. Commercially available enzyme screening kits [18]

Type of enzyme screening kit	Supplier
Alcohol dehydrogenase	Thermogen, Biocatalytics
Esterases and lipases	Altus, Fulka, Roche, Thermogen
Nitrilases	Biocatalytics
Proteases	Altus
Transaminase (aminotransferase)	Biocatalytics

Biocatalysts consist of a catalytic pocket equipped with exactly placed functional groups for catalysis, restricted by protein shells [28]. The progress of enzymatic methods explanation and biotechnology has also help to the popularity of enzymatic methods. Due to possibility of isolate, identify, clone, and over express the genes included in the biosynthesis of benign natural molecules, thereby opening the possibility to use special enzyme in vitro and site-selective mutation if performance needs to be progressed, for example to tailor the enzyme to better or to enhanced or change the enantioselectivity (Figure 2) [29].

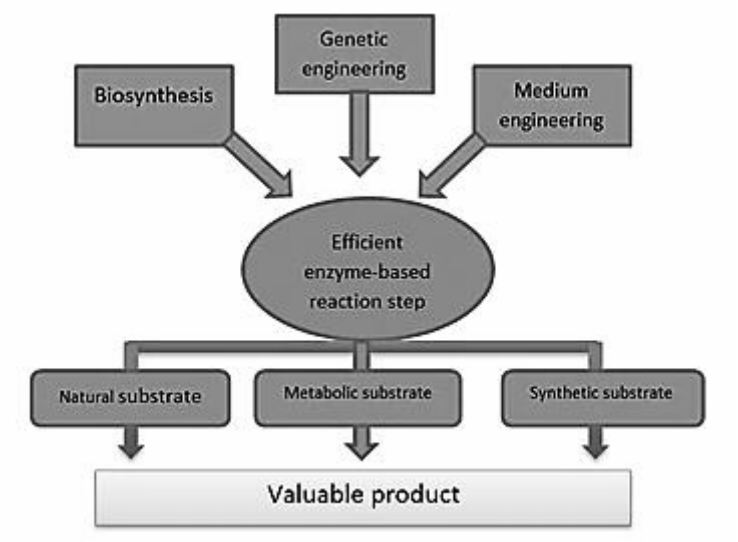


Figure 2. Rationale of the development of an enzymatic reaction step in organic synthesis [29].

3. How Enzymes Work

There are at least 21 various theories for how enzymes can catalyzed organic reactions have been presented. Among all these theories, a common one is that enzymatic processes are usually initiated by the production of an enzyme-substrate structure (or E-S complex), from which the catalysis happens. Originally, the concept of an E-S complex was offered independently by Brown in 1902 [30].

3.1. Lock-and-key hypothesis

This is the simplest model to represent how an enzyme works. An enzyme is the lock into which the substrate (the key) fits (Figure 3). In this model, the active site of the unbound enzyme is complementary in shape to the substrate [30]. Although this interaction among substrate and enzyme will account for a high degree of specificity of enzymes, the observed phenomena does not rationalized by lock-and-key theory. For example, molecules whose structures are referred to that of the substrate, but with less bulky substituents, usually flunk to be substrates, even though they could fit into the biocatalyst. Some molecules with bulkier substituents can bind more strongly to the enzymes than does the substrate. If the lock-and-key hypothesis was correct, one would think that a bulkier molecule would not fit into the lock. Several enzymes that catalyze organic reactions between two molecules do not bind one reactant until the other substrate is already bound to the biocatalyst [30].

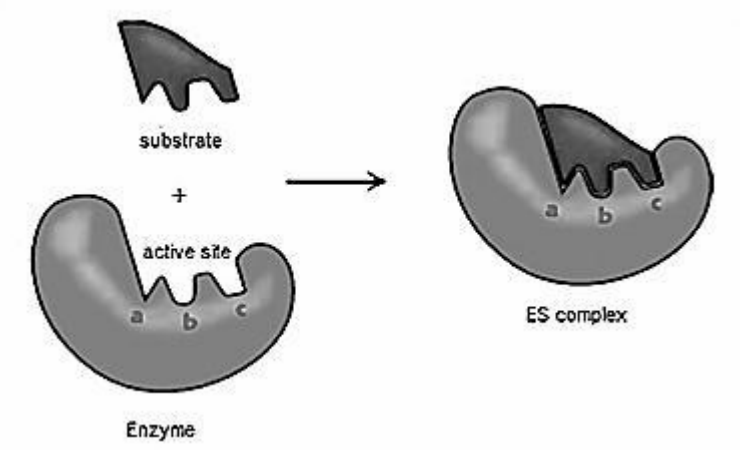


Figure 3. Lock-and-key model of enzyme-substrate binding [30].

3.2. Induced-fit hypothesis

In this approach, the structure of enzyme converts shape as the substrate compounds get closer. When a substrate trends to bind to

enzyme, the interactions of different functional groups on the substrate, with specific enzyme groups are initiated, and these reciprocal interactions cause a conformational change in the enzyme (Figure 4). After the substrates have been bound, the active site forms a shape supplementary to the substrate [30].

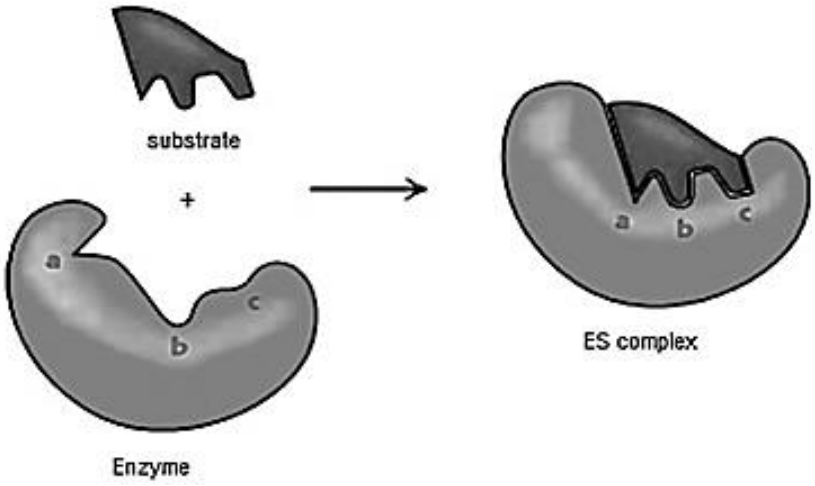


Figure 4. Induced-fit model of enzyme-substrate binding [30].

4. Type of Enzymes

Although the existent enzymes in nature are about 25000, approximately 4000 enzymes have been recognized by The International Union of Biochemistry and Molecular Biology (IUBMB) and categorized using enzyme nomenclature into six categories (Figure 5): hydrolases, oxidoreductases, isomerases, transferases, lyases, and ligases. Among these types, oxidoreductases and hydrolases are the most widely applied biocatalysts in biotransformations reactions. As an example, in the 1987-2003 time periods around 85% of enzyme researches were done on oxidoreductases and hydrolases (25% and 60%, respectively). Table 2 demonstrates some applications of these six categories [18, 31-33].

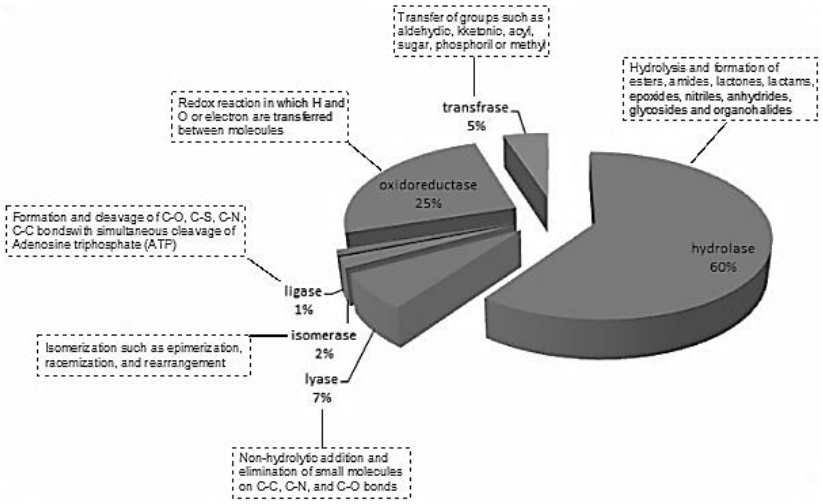


Figure 5. Classification of enzymes [31].

Table 2. Broad classification of reactions that enzymes catalyze [31]

Enzyme class	Reactions catalyzed	Examples
Oxidoreductases	Oxidation or reaction of substrates	Dehydrogenases Oxidases Peroxidases Oxigenases
Transferases	Transfer of a group from one molecule to another	Methyltransferases Glycosyltransferases Acyltransferases
Hydrolases	Bond cleavage while water is added	Esterases Glycosidases Peptidases
Lyases	Non-hydrolytic cleavage of bonds	Decarboxilases
Isomerases	Conversion of one isomer to another (intramolecular rearrangements)	Racemases Epimerases Intramolecular lyases
Ligases	Joining of two molecules at the expense of chemical energy	DNA ligase

Based on many of the enzymes were available commercially, using lipases and esterases for the catalysis of esterification and different hydrolysis reactions was most common and the potential of nitrile hydratases, nitrilases, and epoxide hydrolases, was beginning to be investigated. Reduction of carbonyl compounds, commonly including easily available micro organisms like bakers' yeast, represented about 10% of the activity, on average. These days, lipases and esterases are used routinely to prepare pure and optically active building blocks for the construction of imaginative novel routes to chiral target molecules. Reduction reactions properties prominently, either asymmetric reduction of imines and carbonyl compounds. Herein, the oxidation reactions are featured involve the operation of dioxygenases for providing the stereoselective oxidation of sulphides and the chiral cyclohexa dienediol derivatives. The reactions whose involve carbon-carbon bond forming are carried out by the enzymatic construction of cyanohydrins and the recent day of formation molecule libraries using aldolases. Several modern researhes involving modification of carbohydrate were investigated, while in the 'other' category, the effect of new catalytic antibodies and the employment of laccases are featured [34].

The main social concerns about energy resources and environmental quality have increasingly made enzymatic methods desirable to pharmaceutical industries [35]. Although these six types of biocatalysts are found in nature, more than 80% of industrial synthesized enzymes are hydrolases, of these approximately 50% act on carbohydrates, and also provide most food industry related enzymes [33].

4.1. Oxido-reductases

Redox biocatalysts are easily classified based on the nature of oxidizing reactant (the electron acceptor) or their coenzyme requirement alternatively and the reaction products. These enzymes can be divided into four groups: peroxidases, oxidases, dehydrogenases/reductases, and oxygenases/hydroxylases. Hydroxylases and oxygenases can catalyze the interpolation of oxygen atom(s) into organic substrates, using an oxygen donor like molecular oxygen (O_2). These enzymes divide to two groups:

dioxygenases/hydroxylases which can catalyze the insertion of both oxygen atoms, and monooxygenases/hydroxylases which can catalyze the insertion of a single oxygen atom. While dehydrogenases catalyze hydrogen removal but do not involve active oxygen intermediates, microbial oxygenases, peroxidases, and oxidasesemployed in enzymes; in each of these groups, oxygen atoms as the electron acceptor can be served (Figure 6). In generally, oxidases and peroxidases- as molecular oxygen or peroxide, respectively- react with oxygen, and producing reactive oxygen intermediates that then react further with reducing substrates. Therefore, the reactions are nonspecific and produce various products. Oxygenases are usually more selective than peroxidases and oxidases, especially in the field of regiospecificity due to inducing one or two oxygen atoms into their substrates [17, 36].

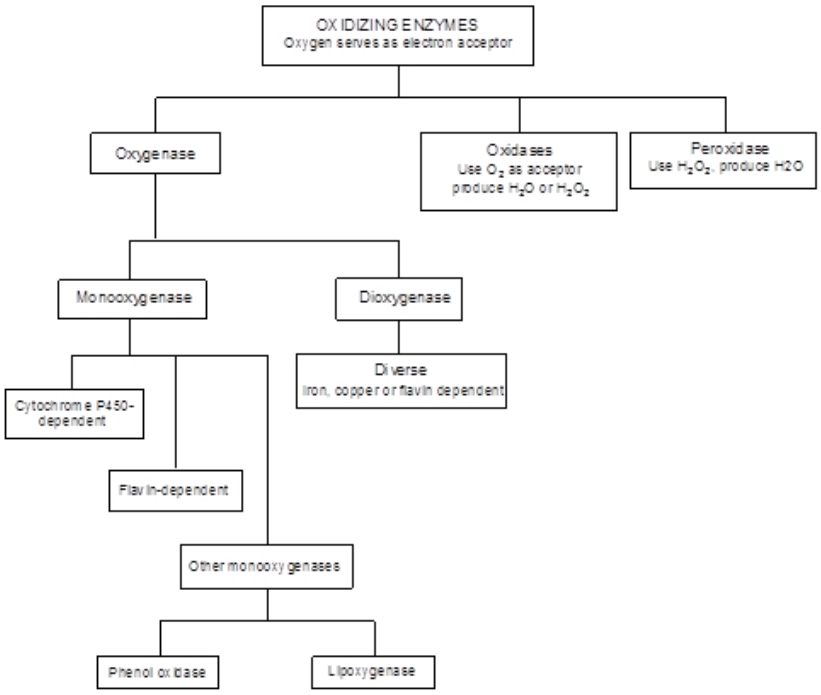


Figure 6. Classification of oxidizing enzymes [17].

4.1.1. Monooxygenases

These enzymes can catalyze the interpolation of a single oxygen atom from molecular oxygen into an organic compound [36]. Monooxygenases employ in organic cofactors to shift electrons to O_2 for its activation. NADH or NADPH is used by monooxygenases to provide reducing potential for the supply of electrons to the substrate molecule and catalyze the insertion of one atom of oxygen into substrate:

$$\text{Substrate} + \text{donor-H} + O_2 + H^+ \longrightarrow \text{substrate-O} + \text{donor} + H_2O. \quad (1)$$

They are highly enantio-, chemo-, and/or regioselective that make them attractive catalysts. This system include an active heme-oxygen structure in which single electron reduction can reduces an iron (III)-substrate complex, and then this complex can reacts with oxygen to produce an iron (II)-substrate-O_2 intermediate; addition of the second electron give water and oxygen addition to the substrate. In monooxygenases which are flavin-dependent, a flavin-oxygen intermediate should react with substrate, producing water and requiring cofactors for production of the flavin moiety. Although the non-P45 hydroxylases are often as a separate group, and catalyze the insertion of a hydroxyl group to replace a hydrogen atom at saturated carbon, are also monooxygenases. Monooxygenase from bacterial cytochrome P450 catalyzes the NADH-dependent reduction reactions of oxygen to a reactive structure can oxidize numerousmolecules, involving polyaromatics, arenes, terpenes, and arylhydrazines. Recently, there have been reported monooxygenase from pseudomonas putida Gpo1 can epoxidize the terminal alkenes, styrene, and enantioselective sulphide oxidations yield chiral sulphoxides in efficient reactions (Figure 7) [17, 36].

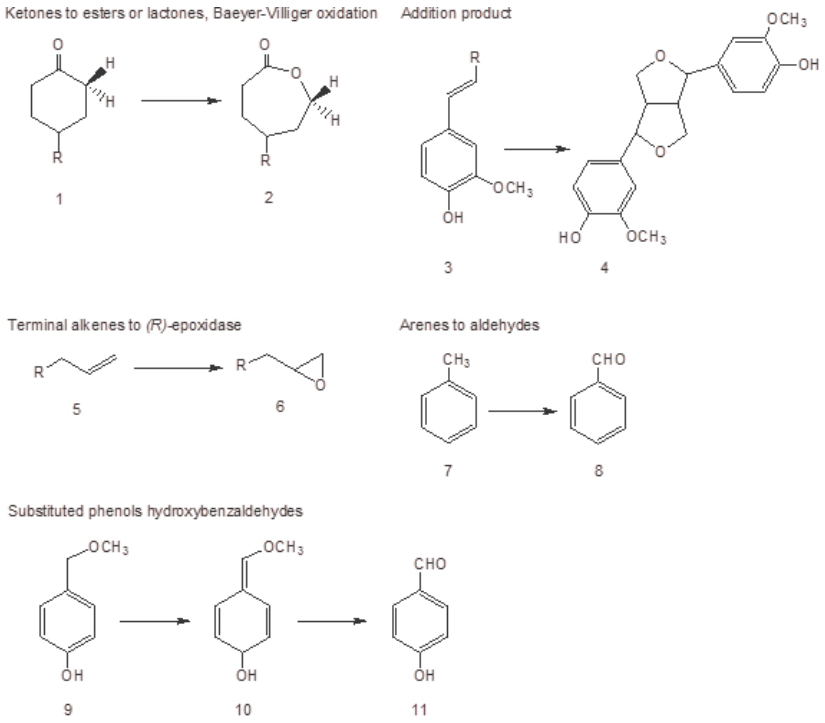


Figure 7. Examples of oxidative biotransformation reactions-monooxygenases [17].

4.1.2. Dioxygenases

Dioxygenases are used to incorporate dioxygen into substrates and are including two major classes: heme-dependent iron-sulphur plant dioxygenases and Rieske iron-sulphur non-haem dioxygenases. The latter are almost NADH dependent and exhibit higher redox potentials. Their reactions involve substrates range from arenes (naphthalene, toluene) to carboxylates (phthalate, benzoate), aromatic ring cleavage, and cis-dihydroxylation and hydroperoxidation. These include the ring-cleaving dioxygenases like catechol dioxygenase that merge both atoms as hydroxyl group on neighbor carbons of aromatic rings, producing cis-dihydrodiols which can be more oxidized to ring-opened products [17].

A wide variety of reactions can be carried out in the enantioselective dihydroxylation of arene, several products are available, and the location of hydroxylation significantly depends on substituents on reactants. Enantio- and regioselective dioxygenase biocatalysis processes can be improved on a rational basis. The important synthons, such as those for prostaglandins and hypotensive agents will be yield from syntheses via these reactions. In microbial systems, the micro-organisms can metabolized the diols further frequently and thus are not accumulated, but the diols are not further metabolized by blocked mutants, and production of cis-diols using *P. putida* is now operated at a commercial scale. Stilbene dioxygenase was applied to cleave vinyl bonds to produce aldehydes, giving a potential path for vanillin generation, isolated from cloned in *E. coli* and *P. putida* TMY1009 (Figure 8) [17].

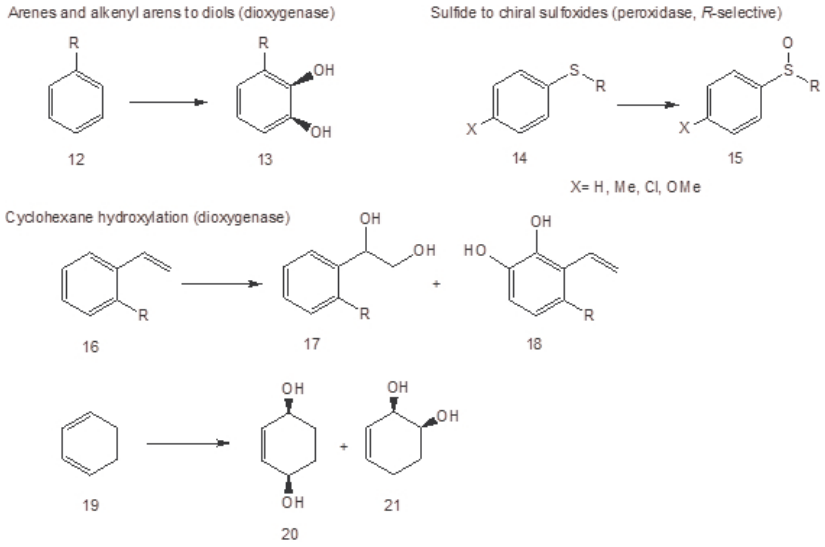


Figure 8. Examples of oxidative biotransformation reactions-diooxygenases and peroxidases [17].

4.1.3. Other oxygenases

A number of oxygenases can separately be synthesized from monooxygenases, involving the copper-containing oxidases. Newly, several novel oxidases are reported. Among them, vanillyl-alcohol oxidase (EC 1.1.3.38) which is a flavor protein, obtained from *Penicillium simplissicum*, active in the biocatalized-oxidation of *p*-substituted phenols, involving vanillyl alcohol to vanillin. It can also catalyze dehydrogenation, hydroxylation, and deamination reactions, and reaction yields include flavor molecules. These enzymes resemble other monooxygenases but are prominented by covalent binding of the flavin moiety in an uncommon binding fold [17].

4.1.4. Peroxidases

Peroxidases are hemoproteins and can catalyze reactions in the presence of hydrogen peroxide. They are produced by a number of micro-organisms and plant sources. Peroxidases are often provided in plant, animal, and microbial cells [17, 26]. They have a molecular weight ranging from 30,000 to 150,000 Da. In plants, peroxidases take part in the lignification reactions and the processes of advocacy in infected tissues or physically damaged [37]. Most of them are heme-proteins with ferric protoheme group related as the prosthetic group, and other cases have magnesium, selenium, flavin, or vanadium group at their active sites. An electron acceptor like hydrogen peroxide is used by peroxidases in the reactions. These enzymes react non-selectively through free-radical paths and can catalyze the oxidation of a various inorganic and organic structures, epoxidations, oxidations, such as hetero-atom oxidation, and reduction of hydro-peroxides in numerous compounds. Peroxidases have typically highest redox potentials and so are strongest oxidizing agents than other oxidases. Because of their high redox potentials, the fungal peroxidases (particularly lignin peroxidase and manganese-dependent peroxidase), and the microbial peroxidases, are the best known as

biocatalysts. Peroxidases from fungal have many advantage over those typically produced extracellularly because their biological effect is in the separation of nutrients by oxidative dissociation of lignin. Among the many useful applications of these enzymes in biocatalysis, the carbon-carbon bonds formation is well known that is coupling reaction. There has been reported the potential for polymer generation from phenols by using these enzymes, but the control or these processes are difficult. The term peroxidase introduces a class of specific biocatalysts, like NADH peroxidase, glutathione peroxidase, and iodide peroxidase, and so a group of non-specific biocatalysts which are easily known as peroxidases [17, 37].

4.1.5. Laccase

Another type of oxidative enzyme, which applied as biocatalysts in the organic reactions named Laccase. They are multi-copper-containing biocatalysts that can produce water by reduction of molecular oxygen and coincidently operate one-electron oxidation of different molecules like aliphatic and aromatic amines, methoxy-substituted monophenols, and diphenols. These enzymes have been obtained in prokaryotes, insects, higher plants, and fungi. Recent reports covering their repartition have certified the ubiquitous nature of them. These enzymes have previously been ignored, due to they were not commercially accessible. Newly, they are used in the textile, and pulp & paper industries due to efficient and environmentally desire reactions; as a result, the use of laccases have enhance attracts in these essentially 'green' catalysts, which operate with air and generate water as an byproduct, making them more generally useful for scientists. Consequently, there have been published a significant number of reports in the recent years, which have investigated the biochemical properties of laccases and/or their applications in bioremediation and technological processes in addition to their use in chemical reactions. Laccase catalyzes the reaction of electron reduction of O_2 to H_2O, conjugate with the single-electron oxidation of four hydrogen-releasing molecules. This was carried out by a cluster of

four copper atoms (type 1 copper; type 2 copper; and two type 3 copper atoms) which produced the active site of enzyme. Type 1 copper, which admits the blue color to biocatalysts because of severe electronic sorption of coppercysteine bond, is the site that monoelectronic oxidation of molecules happens. Type 2 and type 3 copper atoms generate trinuclear clusters, to which the electrons can be transferred and cause to molecular oxygen reduces to water. The special result is the oxidation of four substrates of suitable molecules to generate four radicals since reducing one oxygen molecule to two water molecules. Afterwards, the reactive radical can done several non-biocatalytic processes, which involve (i) covalent coupling to produce polymers, oligomers, and dimers, via C – N, C – O, and C – C, bonds; (ii) cleavage of covalent bonds to degradepolymers, especially alkyl-arylbonds (often in the presence of mediators); and (iii) ring cleavage of aromatic molecules [38, 39].

These enzymes cannot oxidize such substances and the reported redox potentials of laccases are lower than those of non-phenolic compounds. However, it was shown that the oxidation of non-phenolic compounds can also be done by these enzymes in the presence of small molecules which electron transfer mediators. Laccase-mediated systems (LMS) have been functioned to many reactions like oxidation of organic pollutants, pulp delignification, and improvement of biofuel or biosensors cells. Herein, numerousinorganic and organic structures have been reported as an effective mediator for the mentioned aims [40]. Based on ability to oxidize different aromatic molecules, the applications of these enzymes have been explored, include: (i) drugs analyses (biosensor to discern codeine and morphine); (ii) textiles bleaching; (iii) water/soil reconstruction and green chemistry in order to protect the environment from hurts caused by urban or industrial pollutants; and (iv) modification of wood fiber in the pulp and paper industry [41].

Because of their advanced reactivity with considering reaction safety, selectivity, health, and environment aspects, catalyzed reductions and oxidations by redox enzymes have prospered to the implements of choice (Figure 9). Since traditional oxidations (chemical methods) usually employ excess stoichiometric oxidants, the selective elimination of residual oxidants is crucial for quality of products and biocatalytic approaches have become standard methods in production. Selective presentatin of one or more oxygen atoms by enzymes has adsorbed many industrial attentions. Among the processes introducing one oxygen atom, inexpensive organic building blocks are oxyfunctionalization by selective asymmetric Baeyer-Villiger oxidations, epoxidations, and hydroxylations, which are interesting and significant progress. Among the last decades, selective enzymatic oxidations of one out of various hydroxygroups generally in sugars and alcohols continue to be of industrial attentions and have extra sustainability benefits over the traditional chemical oxidations [42].

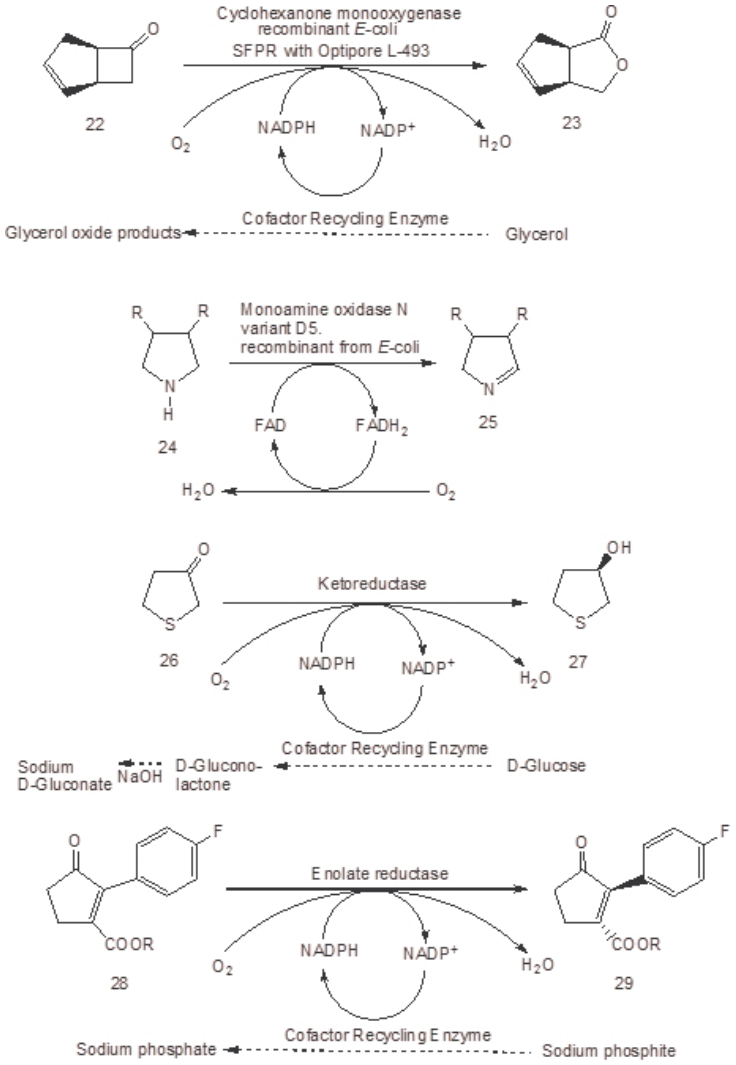


Figure 9. Selected biocatalytic oxidation and reduction reactions [42].

Laccases have been studied in various fields, such as applicability in molecular genetics, genetic transcription, genetic expression, and cloning. Laccase oxidizes many substrates: phenolic dyes, phenols, chlorophenols, *N*-substituted phenylenediamines, lignin-related diphenylmethanes, organo-phosphorus, benzopyrenes, and non-phenolic beta-O-lignin model dimer [26].

4.1.6. Hydroxynitrile lyases

Based on the presence or absence of flavin adenine dinucleotide (FAD), the hydroxynitrile lyases are classified into two major groups: Hydroxynitrile lyase I (HNL I) and Hydroxynitrile lyase II (HNL II) (Table 3). This cofactor provides structural stability to enzyme structure or it is present as an evolutionary remnant and is not involved in net redox reaction, but their removal causes inactivation of the enzyme, i.e., the FAD generally binds covalently to a hydrophobic region near the catalytic site of enzyme [43].

Table 3. Characteristics of hydroxynitrile lyases I and II [41]

Characteristic	HNL I	HNL II
Cofactor	FAD containing	FAD lacking
Glycosilation	*N*-glycosilated	–
Chiral glycon	Mandelonitrile	Acetone cyanohydrine, (*S*)-Mandelonitrile, (*S*)-4-hydroxy mandelonitrile, (*R*)-2-butanone cyanohydrins
Cyanogenic glycosides	*R*-prunasin and *R*-amygdalin	(*S*)-Dhurrin, (*S*)-sambunigrin, linamarine, lotaustraline
Carbohydrate content	30%	9%
Isoforms	Presence of isoenzymes	–
Homology	Oxidoreductases (30%)	a/β Hydrolases, serine carboxypeptidases, Zn^{2+}containing alcohol dehydrogenase
Molecular weight	50-80kDa	28-42kDa
Isoelectric pH	4.2-4.8	3.9-4.6

In a study, the hydrocyanation of ketones and aldehydes was carried out to synthesis of chiral cyanohydrin by using hydroxynitrile lyases obtained from almond. The enzymatic asymmetric condensation of HCN with ketones or aldehydes is used to generate cyanohydrins (Figure 10). On the same study, the (*S*)-hydroxynitrile lyases from *Hevea brasiliensis, Manihot esculenta,* and *Sorghum bicolor*, and (*R*)-hydroxynitrile lyase from *Prunusamygdalus* have been used as biocatalysts for the enantiospecific addition of hydrogen cyanide to aldehydes [43].

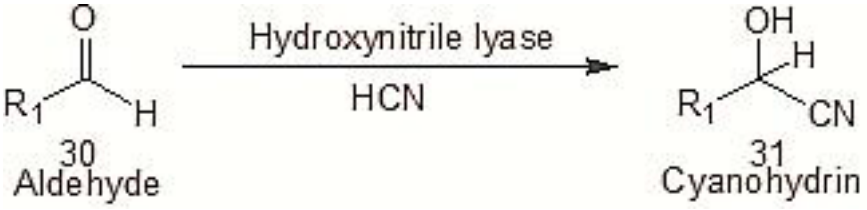


Figure 10. General reaction catalyzed by hydroxynitrile lyase [43].

The enantioselective C – C-bond formation can be done by these enzymes catalyze through the addition of HCN to ketones or aldehydes to give the desired asymmetric cyanohydrins (Figure 11), that synthetically are versatile building blocks for production of nonhazardous chemicals, agrochemicals, and pharmaceuticals. Both *(S)*-and (*R*)-selective HNLs are widely found in the nature, and various genes have been expressed and cloned in micro-organisms like *P. pastoris* and *E. coli*. Biocatalysts that are used in commercial organic reactions, however, are partially purified productions or typically crude cell lysates to reduce the highcosts of the enzyme [5].

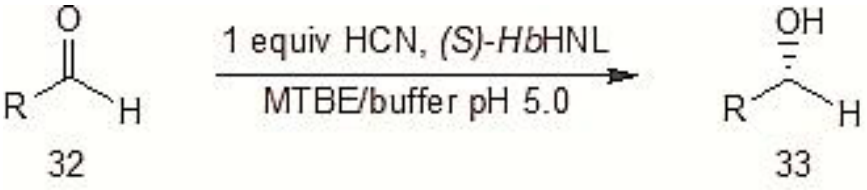


Figure 11. General enzymatic synthesis of (*S*)-cyanohydrins [5].

4.2. Hydrolases

Hydrolases are an important group of biocatalysts. They are classified based on the type of chemical bond that is active on. These biocatalysts show a digestive bio catalytic activity in the nature [8]. Hydrolases can catalyze the hydrolytic cleavage of C – C, C – N, C – O, and some other bonds, involving P – O bonds in phosphates (especially in fat and oils). Their wide applications are including esterification of fatty

acids, hydrolysis of nitriles, polysaccharides, lipids, and proteins. The term hydrolase is involved in the systematic name, which firstly includes the name of the molecules and then the suffix-ase [18, 44]. However, their catalytic activities are more heterogeneous, and many organic transformations are carried out by these biocatalysts. These enzymes, especially lipases, exhibit various benefits over other enzymes, as they have desire activity in aqueous and non-aqueous organic solvents, allowing the transformations of non-aqueous soluble molecules; they need no cofactors for their catalytic activities; moreover, lots of hydrolases are commercially available and handling of enzymes activity in easy; they usually show low substrate specificity and, using these biocatalysts has obtained more attentions for synthesizing of biologically active structures and chiral building blocks in last decadebeca use of the high enantio-, chemo-, and regions electivity of these enzymes (Figure 12) [8].

Using hydrolytic biocatalysts in organic synthesis is common. The applications of these hydrolytic enzymes in reverse hydrolysis processes usually rely on their stability and activity in non-aqueous media that benign product generation [45].

Hydrolytic enzymes, especially proteases and lipases, which have successful applications in organic syntheses, can be generally applied for synthetic processes by employing high concentrations of reactants or removing as much water as possible from the reaction system [46].

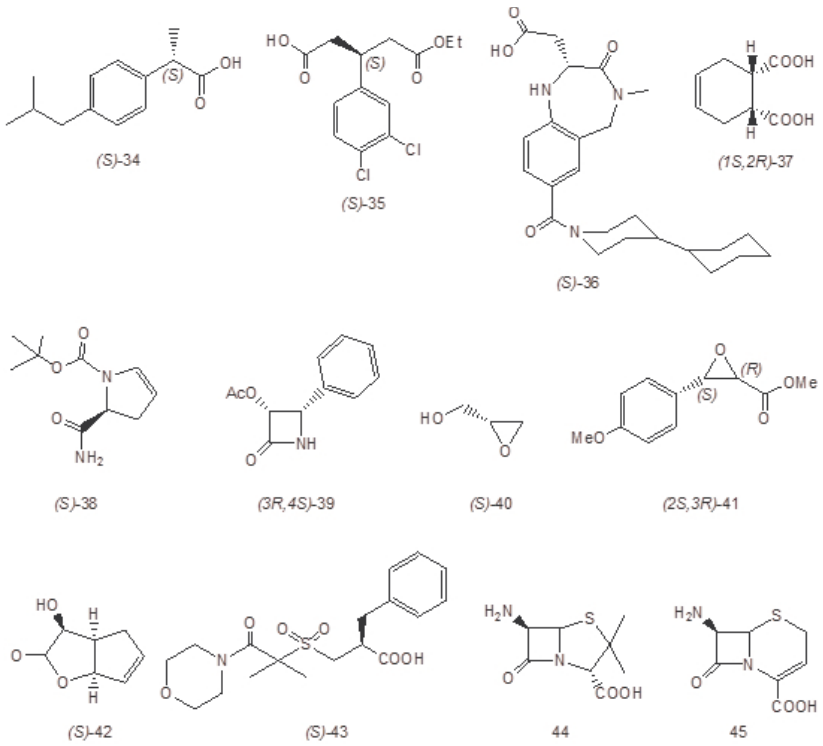


Figure 12. Some chiral building blocks obtained via hydrolase catalyzed-biotransformations [8].

4.2.1. Nitrile hydratases

The hydration of nitrile to expected amide is catalyzed by nitrile hydratases [47]. Nitrileis widely applied intermediates and starting reactants in organic reactions. Herein, the hydration of nitrile to desired carboxamide is a crucial process in organic reactions and nature. There are two methods for hydration of nitriles: enzymatic hydration by nitrile hydratase (Nhase) and chemical hydration. To produce organic acids, bio transformations of nitrile follow two approaches. First, nitriles are hydrolyzed to generate the corresponding ammonia and carboxylic acid by the effect of nitrilase. In another approach, Nitrile is firstly hydrated into amide by Nhase and then, amideis hydrolyzed to ammonia and carboxylic acid by amidase activity. As shown in Figure 13, these three

enzymes (amidase, Nhase, and nitrilase) are the most important biocatalysts in the nitrile metabolism. The enantioselective-transformation of nitriles can be achieved by non-enantioselective Nhases in combination with highly enantioselective amidase [48].

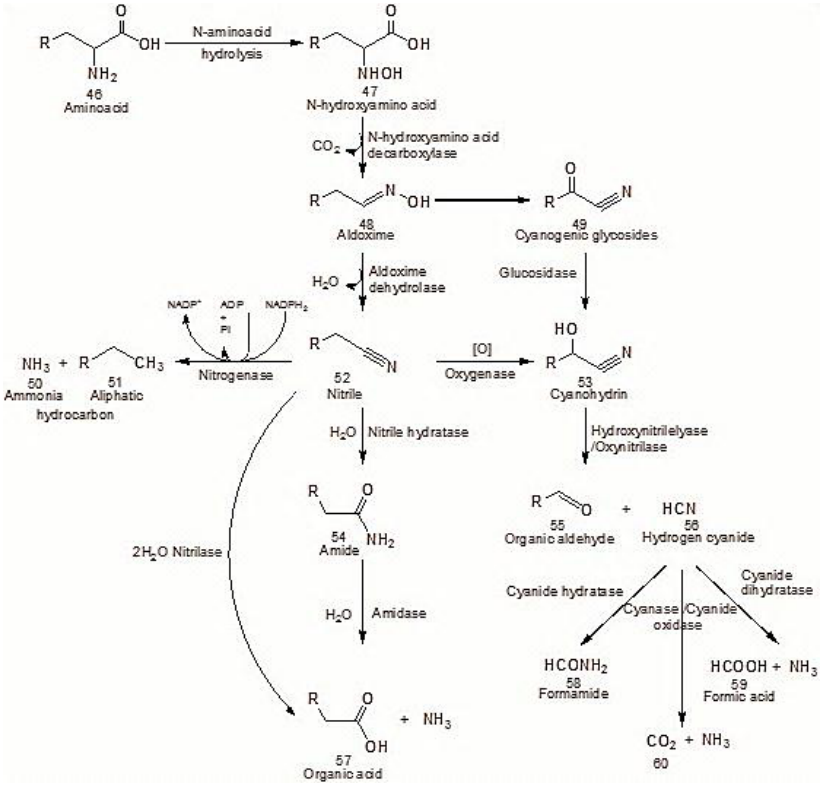


Figure 13. Metabolic pathways for nitrile synthesis and degradation in micro-organisms [48].

Among the reported Nhase preparing micro-organisms, some strains of *R. rhodochrous*, *Rhodococcus* sp., *Nocardia* spp., and *P. chlororaphis*, have been widly investigated and extensively applied for the production of various amides like, nicotinamide, acrylamide, pyrazinamide, butyramide, 3-indolacetamide, isonicotinamide, 2,6-difluoro-benzamide, picolinamide, 2,6-dichloro-benzamide, benzamide, 2-thiphen-ecarboxamide, 5-cyanovaleramide, and indole-3-acetamide by using related nitriles. Out

of these group of organic amides, the first prosper example of a biotransformation reaction is Nhase-mediated production of acrylamide used for the production of merchandise chemicals and also the first case in which, biotechnology approaches were used in the petrochemical industries [48].

Nhase has been employed at industrial scale for generating of various chemicals such as acrylamide. The biotransformation of 3-cyanopyridine to nicotinamide by using Nhases has imbibed increasing intrests as an advanced and useful method, due to the high selectivity of enzymes under nonhazardous mild reaction conditions in aqueous media (Figure 14) [49].

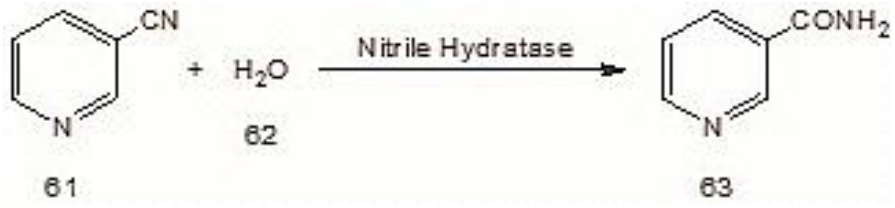


Figure 14. Enzymatic preparation of nicotinamide from 3-cyanopyridine [49].

4.2.2. Amidase

Amidases are a group of hydrolytic biocatalysts that can catalyze the reaction of hydrolysis of amide to carboxylic acid. There are many researches have shown that amidases obtained from varoius micro-organisms demonstrate a wide substrate range and high enantioselectivity against racemic molecules. Thus, the kinetic resolutions of a wide variety of racemic amides have been catalyzed by amidases. Surprisingly; the selective de-symmetrization of prochiral diamides amidase has remained largely unexplored [50].

The prominent effect of amidases would be to continue likely at high temperatures, in the presence of other alternate Nhase-susceptible structures. Up to now, during the pre-cultivation, the Fe ion has obtained

a very high activity of Nhase. When Fe ions are in pre-cultivation, the selective conversions of a mixture of 2-naphthylacetonitrile (64) and 2-naphthylacetamide (66) were prosperous (Figure 15). Under the conditions at 3.8% (v/v) addition of DMF and 45°C, the Nhtases were completely deactivated, and 99% of the nitrile (64) was recuperated. In addition, amidases worked well to produce desired acid (67) in 99% yield [47].

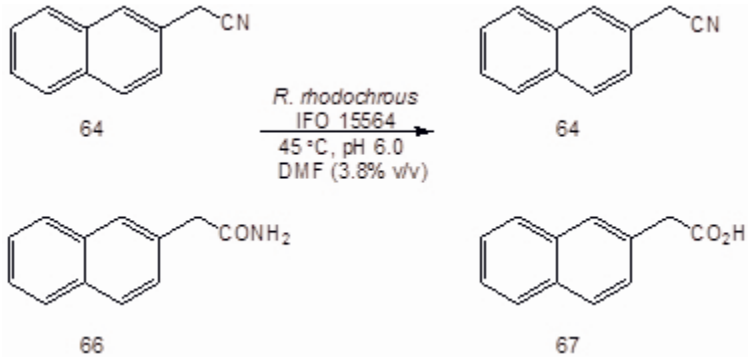


Figure 15. Preferential action of amidase of *R. rhodochrous* IFO 15564 on the mixture of a nitrile and an amide [47].

4.2.3. Lipases

Lipases (triacylglycerol ester hydrolases) are ubiquitous enzymes [51-55]. They are mainly divided into general three groups chosen as subfamily I.1, I.2, and I.3, based on molecular features and amino acids trail homology. Many *Pseudomonas* lipases like Amano P, P-30, lipase AH (from *Bacillus cepacia*) have been commercially available, lipase 56P, and lipase AK (obtained from *Ps. Fluorescens*). They show significant activity in organic reactions for high scale of valuable chemicals. There are many crystal structure designation of various lipases have been reported simultaneously involving lipases from *Ps. Aeruginosa* (group I.1), *B. cepacia* (group I.2), and *Ps.* Sp. MIS38 (group I.3). Protecting of lipases is vital for folding to bio catalytically active; therefore, *E. coli* is adsorbed great attention of organic chemists and industrialists to

enhance lipase efficiency in economic expression and bio chemically safe systems. In addition, lipases are strongly hydrophobic enzymes in nature. These enzymes generate toxicity to host cells, forming inactive inclusion bodies, or leading to their death, when frequently aggregated as foreign protein structure in cytoplasm [56].

Enzymes including lipases have been widely investigated in recent decades. Over the enzymes, lipase is the most frequently enzyme used in organic syntheses. Applications of microbial lipase has become very interesting for industries because of their widely various enzymatic features particularly catalyzing the formation of a wide spectrum of amide and ester bonds, and the ability to operate enantioselective hydrolytic mechanisms. They are also activated when attach to an oil-water interface and can be active and stable even under high alkaline pH (the optimized pH 10) [52, 53, 57, 58].

High stereo-, regio-, and chemoselectivity have commonly made this enzyme applied enzyme in synthetic chemistry [59-61]. Lipases have demonstrated to be better enzyme for operating numerous organic mechnisms like transesterification, esterification, organic reactions in water restricted media, and stereospecific hydrolysis of racemic esters [62]. Reactions which use lipase are done in emulsion systems that the reaction occurs at the interface of small oil droplets. Lipases can successfully catalyze reactions in an organic medium under almost anhydrous conditions; the range of suitable reactions is more than hydrolysis reactions. Moreover, lipases were instrumented to catalyze the reverse reactions of organic synthesis in low aqueous media. Advantages of using lipase in organic reactions, such as short chain ester production including fragrance and flavour structures, have long been investigated. Various studies concerning bio transformations have been reported in literature [2, 60, 63]. Microbial lipases which can apply as biocatalysts in non-aqueous media present novel approaches, like improving thermal stability of the enzymes, shifting of the thermodynamic equilibrium in favor of productions, enabling the use of hydrophobic reactants, and engineering solvent to control specificity of substrates. Various reactions,

such as esterification, transesterification, hydrolysis, alcoholysis, amidation, or acidolysis, hydrazinolysis, lipophilization, and epoxidation can be done in non-aqueous media by these kinds of enzymes. Lipases are commonly chosen as biocatalysts for the synthesis of chiral compounds, via the kinetic resolution (KR) of racemic mixtures or the enantioselective enzymatic desymmetrization (EED) of prochiral compounds [8, 31, 53, 62, 64-66].

Owing to specificity of lipase substrates, such as region-stereospecificity, alcohol, and fatty acid, lipases are markedly applied in various fields of medicinal, pharmaceutical, biotechnological, hydrolysis of oil and fats processing, surfactant, textile, dairy, detergent, and food [67, 68]. Lipase catalyzed selective acylation/deacylation reaction presents a notable role in enzymatic transformations in organic reactions, which is generally related to low cost of lipases and their high endurance against various substrates and reaction conditions [9]. Amides can also be synthesized from non-activated esters by lipases. Primary amines with ethyl butyrate are acylated by *Candida antarctica* lipase (CAL) that exhibit a very high activity and specificity. N-octyl alkylamides and fatty acid amides have been synthesized in anhydrous hexane and using *Mucor miehei* lipase, respectively. Synthesis of benzoic acid hydrazide was conducted by employing different lipases, amongst which Novozym 435 was found to be the most active catalyst. Different lipases are used for synthesis of fatty acid analogues of capsaicin and amongst them *Pseudomonas cepacia* lipase was the best [69].

Lipases are prominent biocatalysts with capable to catalyze many reactions in non-aqueous and aqueous solvents. Lipase has a pretty abstruse catalytic behavior. In aqueous homogeneous systems, they are in equilibrium between two structural situations: (i) “closed” or inactive state, in which a helical lid covered the catalytic triplet in the active site; and (ii) “open” or active state, in which the lids have been replaced, adopting a generally alternate conformation and showing the catalytic rests. While the drops of natural molecules (oils) in the reaction condition, lipases will bet imbibed to hydrophobic site through the very hydrophobic zone obtained by the area surrounding the active face and

the internal site of the displaced lid. This lid replacement and other entailed conformational converts finally result in both shaping the catalytic machinery (induced fit) and providing space in the active site for lipid linking [60, 70, 71].

Lipases are quite stable in non-aqueous solvents and a wide range of substrates could be accepted by these types of enzymes [72]. Lipases are inexpensive, environment-friendly biocatalysts for triacylglycerols hydrolysis, and are applied in mild condition [73]. These enzymes can practically be redefined as carboxylesterase activating on long-chain acylglycerols, when they are termed after various type of processes they can catalyze; on the other hand, lipases are simply fat-breaking 'ferments'. Herein, they are usually named triacylglycerol hydrolases. Since the processes are reversible, the productions of acylglycerol from free fatty acid and glycerols also catalyzed by lipases (Figure 16) [2].

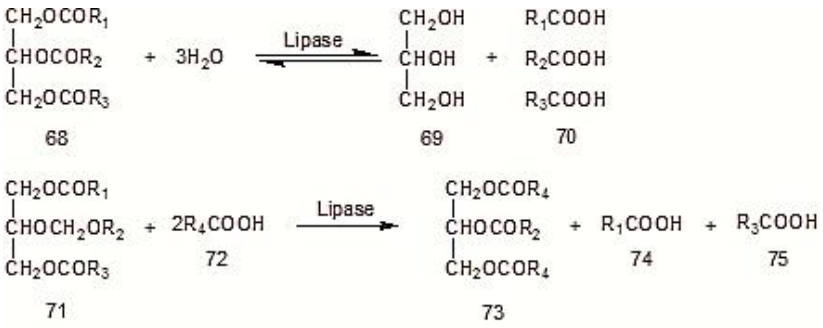


Figure 16. Examples of lipase-catalyzed reactions of triacylglycerols [2].

A lipase catalyzes a condensation reaction (reverse hydrolysis) to produce various esters, such as polyesters and polyol esters, soluble or insoluble carboxylic acid esters, fatty acid esters, and aliphatic alcohol esters (Table 4). Lipases almost are used in organic synthesis like catalyzing the chemo-, regio-, and/or stereoselective hydrolysis of carboxylic acid esters or the reverse reaction in organic solvents. Lipases especially *Candida antarctica* Lipase B (CALB) is also widely used in polymerization reaction due to ability to function in aqueous and organic

reaction environments. Various reactions have also been carried out by CALB, such as transesterification and alcoholysis, for the production of optically active compounds and biodiesel fuel well as for the breakdown of fats, oil, and amides. The high regio- and stereospecificity of lipases cause their various applications: surfactants and biofuel, synthesis of fatty acid esters as cosmetic ingredients, hydrolysis of oils and fats, and production of intermediates for organic synthesis [19, 50, 51, 55, 74, 75].

Table 4. Synthesis of esters by lipase-catalyzed condensation reaction in organic solvent systems [74]

Alcohol substrate	Acid substrate
Glucose	Fatty acid
Glucose	Vinylacetic acid
Fructose	Fatty acid
Mannose	Fatty acid
Galactose	Fatty acid
Methyl-α-D-glucoside	1-Bromomyristic acid
Butyl-α-D-glucoside	Fatty acid
Octyl-α-D-glucoside	Cinnamic acid
Hydroxypropyl cellulose	Fatty acid
Glycerol	Fatty acid
Erithritol	Fatty acid
Xylitol	Fatty acid
Sorbitol	Fatty acid
Ribitol	Fatty acid
Ascorbic acid	Fatty acid
Kojic acid	Fatty acid

The features and structure of lipase are highly influenced by their origin. Many of them from different sources have extensively been used in production of polyester, e.g., *Pseudomonascepacia and porcine* pancreas, *Candida rugosa* lipases, *Pseudomonasfluorescens* lipase, and *Candida antarctica* lipses. Lipase B from *C. antarctica* (CALB), such as Novozym 435, immobilized on Lewatit, has been the most widely studied biocatalysts for production of polyester, and has demonstrated significant activity towards a wide spectrum of monomers [76].

On the other hand, while proteases (EC 3.4) catalyze the transesterification of carbohydrates by using activated esters as substrates, the esterification of carbohydrates has been catalyzed by lipases (EC 3.1) in reversed hydrolysis reactions [77].

Lipases were used to the biocatalytic acetylations of different carboxymethyl celluloses (CMC) with vinyl acetate. The yield of reaction significantly enhancedby increasing lipase amount from 0 to 1000 units, especially with enhancing up to 5000 units per gram carboxymethyl celluloses. A few enzyme active sites (e.g., < 1000 units) provided a bit amount of this enzyme. Figure 17 exhibits various final acetylation yields at the 114h of reaction time, informing that the reaction time may not be enough to give the processes in equilibrium. Figure 17 also demonstrates an obvious difference in tendency of time course profile between the non-enzymatic and enzymatic processes. For the reactions that catalyzed by lipase, the acetyl amounts were initially increased to maximum and then dwindled, which exhibited a characteristic kinetically-controlled reaction course (Figure 17 (2)) [78].

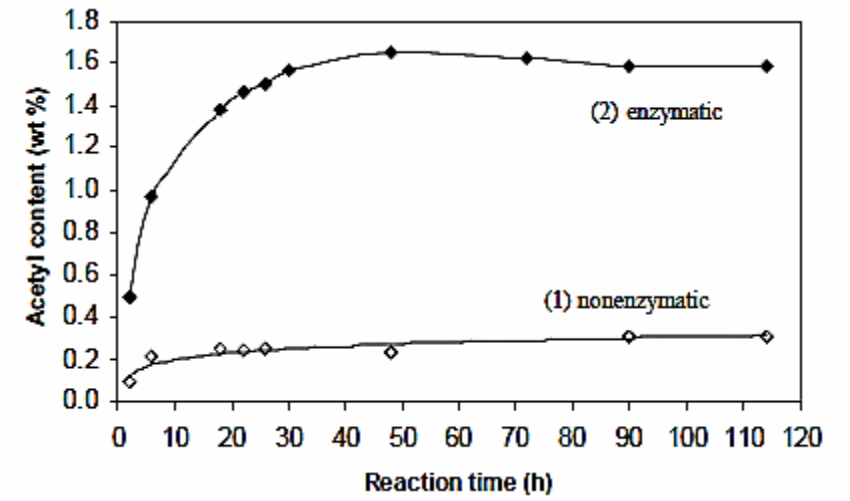


Figure 17. Time courses of CMC acetylation in aqueous medium with and without enzyme [78].

These ubiquitous enzymes have been successfully applied in these kinds of processes, for enantioselective transesterification of secondary alcohols and amines or for enantioselective hydrolysis of esters and amides, giving the desired chiral amides or esters, respectively. Lipases are often the first choice for the synthesis of chiral molecules, through the enantioselective enzymatic desymmetrization (EED) of prochiral molecules or the kinetic resolution (KR) of racemic blends [8].

Lipases can also catalyze synthesis of esters and transesterification processes with high stereo and region selectivity in non-aqueous media. The hydrolysis of medium- or long-chain triglyceride to glycerol and fatty acids can also be catalyzed by lipases, and they are more individuated from esterase in their strongly activation at the interface formed by insoluble lipid molecules in aqueous systems. These properties cause lipases have a key role in the fields of fine chemicals synthesis, agrochemical productions detergent, pharmaceutical, and additives [79]. Lipases of triacylglycerol hydrolases are a group of biocatalysts, named as carboxyl esterases, catalyze the synthesis and hydrolysis to form ester

from the long chain fatty acids and glycerol at the lipid-water interface. Within hydrolysis, lipase picks acyl functional group from glyceride forming lipase-acyl structure, which afterwards transfers its acyl group to hydroxyl group of water [68].

4.2.4. Penicillin acylase

For more than three decades, penicillin acylase (penicillin amidohydrolase E.C. 3.5.1.11) has been applied to synthesis 6-aminopenicillanic acid (6-APA) from the hydrolysis of penicillin V or G. Many microorganisms (fungi, bacteria, and yeast,) can produce penicillin G acylase (PGA) (E.C. 3.5.1.11). PGA applied for catalyzing the formation and cleavage amide bond, especially for β-lactame synthesis. It can also catalyze the synthesis of β-lactam nuclei and suitable acyl donor derivatives by using both kinetic and thermodynamic approaches. It is not supposed that PGAs have activity against vinyl acetate; owing to they are specific for various phenylacetic derivatives. Thus, N-methylimidazole successfully reconstructed the activity of PGA to vinyl acetate that depicted especial effects of N-methylimidazole on biocatalysts. Using small molecules can altered the catalytic activity of acylases from amidation to esterification. The use of penicillin acylase biocatalysts are limited to low product yields and process conditions due to low stability, has obstacled the widespread prosper of biocatalysts in the production of β-lactam antibiotics [8, 80, 81].

It was reported the conflation of biocatalysts activity by organic substrates. The addition of 10% N-methylimidazole increased the esterification activities of PGA more than 70-fold. As seen from Table 4, N-methylimidazole or IPA (PGA immobilized and optimized on acrylic resin from *Escherichia coli*) demonstrated no catalytic activity to esterification among guaifenesin and vinyl acetate (Figure 18) and only modicum yield was observed (entries 1 and 3, Table 5). However, in the presence of 10% N-methylimidazole, the esterification reaction can successfully be proceeding under the catalysis of IPA. The reaction rate

was enhanced over 70-fold and the product was increased up to 84% (entry 2, Table 5). Therefore, to acknowledge which was in charge of the mechanism, some reactions were offered. The esterification could not be catalyzed by BSA in the presence of N-methylimidazole (entry 4, Table 5), which removed the catalysis of the amino acid on the active site of protein. The rate decreased sharply, when converted IPA (pre-treated with urea at 100^{o}C for 16h) was appliedthat ruled out the probability which the support catalyzed the reaction (entry 5, Table 5). The immobilized PGA may haveexcellent chemical stability. Therefore, in the presence of N-methylimidazole in the reaction, the enzyme after inactivation could recover activity and exhibit partial catalytic ability. In addition, when the amount of IPA increased, the reaction rate was improved (entry 6, Table 5). On the contrary, phenylacetic acid and penicillin G can be strongly inhibited the reaction, which was known as IPA inhibitor (entry 7, Table 5). All the above data's showed that the process was a bio catalytic reaction. Due to the enzyme did not exhibit good activity towards PG and the synthesis of β-lactam could not be catalyzed; it should be noted that the amidation operations of PGA were repressed in the presence of N-methylimidazole [80].

Using process designs have also been ameliorated the preparation of the antibiotics 7-ADCA and penicillin G. Recently; one-pot synthesis approaches for dTDP-4-keto-6-deoxy-glucose are carried out by the combinatorial use of enzymes, which is applied as a pioneer for the production of antibiotics. The one-pot approaches led to removed complex and exhausted synthetic steps, increased productivity, and decreased the cost of process [82].

Table 5. Effect of different catalysts[a] on esterification between guaifenesin and vinyl acetate [80]

Additive	Yield (%)	$V_0(\mathrm{mMh^{-1}})$	Vr^g
None	1.8	0.04	1
N-methylimidazole	84.1	2.90	72.5
N-methylimidazole[b]	1.8	0.04	1
N-methylimidazole[c]	2.4	0.05	1.3
N-methylimidazole[d]	26.1	0.59	14.8
N-methylimidazole[e]	84	3.48	87
N-methylimidazole[f]	4.2	0.09	2.3

[a]25mg PGA, 0.2ml additive, 1.8ml acetone, 50°C.

[b]Without PGA.

[c]BSA.

[d]Denatured-PGA.

[e]50mg PGA.

[f]PGA with inhibitor.

[g]Relative initial reaction rate to the reaction in absence of additives.

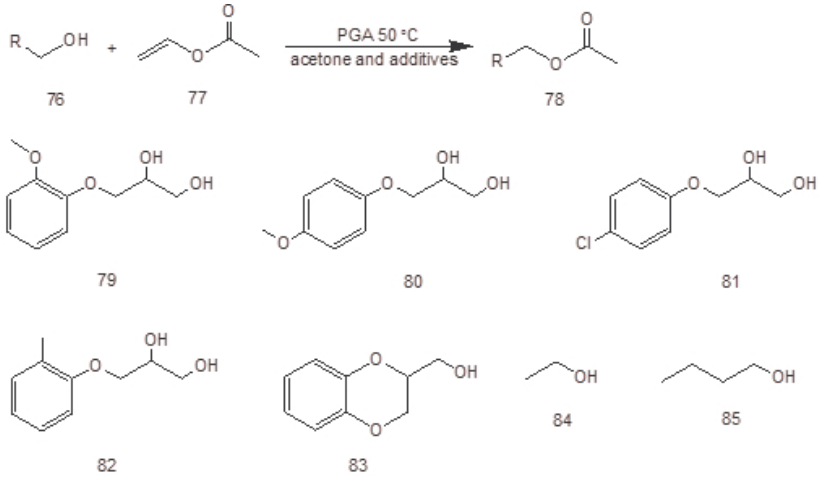


Figure 18. N-methylimidazole manipulates PGA to catalyze esterification [80].

4.2.5. Proteases

Proteases are too important in the industrial reaction sowing to their applications in the chemicals, food, leather silk, pharmaceuticals, detergents, and paper and pulp, industries [82]. They are generally classified into two categories: endopeptidases, which cleave peptide bonds within the protein, and exopeptidases, that cleave off amino acids from the ends of the protein chain. They are a lot of industrial biocatalysts that form over 65% of the world markets. These biocatalysts are widely employed in the textile, food, leather, and pharmaceutical industries [3]. In recent decade, proteases have absorbed an extremely interest in different organic synthesis, because there are various benefits related with the application of proteases for the synthesis of esters and peptides [83]. The hydrolysis reaction can be catalyzed proteases under normal aqueous conditions, but the reverse actions of proteases have been found in water-restricted media, such as synthesis of esters and peptides [84]. Urease (URE) is a highly efficient enzyme, which catalyzes the hydrolysis of urea to ammonium and carbon dioxide. Trypsin (TRY) belongs to the group of serine proteases hydrolyzing peptide bonds in which the carboxyl groups are contributed by the lysine and arginine residues with applications in hydrolysis of protein and detergent industries [85].

The division coefficient of used solvent in an equivalent combination of water and octanol is defined by the parameter LogP (solvent polarity). Numerous organic media with the LogP value equal to or less than 2.0 was chosen for sieving protease with extensively solvent-stability, because hydrophilic media have more tendencies to tape strongly bound water from the biocatalysts structures than hydrophobic ones. The potential producer strains PT121 were chosen for more research due to the favorable stability in the presence of hydrophilic organic media. Proteases are completely stable in the presence of 50% (v/v) organic solvents with the LogP values over 2.0 of incubation for 14 days, and it was less stable in several hydrophilic solvents $(\text{LogP} \leq 2.0)$ except DMF, acetone, isopropyl alcohol, and ethanol (but the residual protease activities still existed after incubation for 5 days). As compared with the

control, benzene (LogP = 2.0) and DMSO (LogP = −1.35) however, were exceptions among hydrophilic solvents, which caused no loss in activity, because the correlation between enzyme stability and solvent polarity (LogP value) could not be well established recently [84].

Natural and non-natural peptides have a key role in the fields of medicine and health care, pharmaceutical and food applications, cosmetics, and nutrition. These peptides can be produced enzymatically for example by catalyzed conjugate of an amino acid amides with protease and a chemically synthesized C-terminally activated N-protected amino acid, e.g., an N-protected carbamoylmethyl (Cam) ester [85, 86]. The enzyme-scissile linker groups were investigated to cleave selectively by proteases, such as subtilisin, chymotrypsin, and trypsin. A-chymotrypsin was used to cleave oligosaccharides or glycopeptides from a solid support at PH 7. This protease recognizes phenylalanine and cleaves a corresponding ester bond of a hexaglycine spacer selectively in high yield (Figure 19) [87].

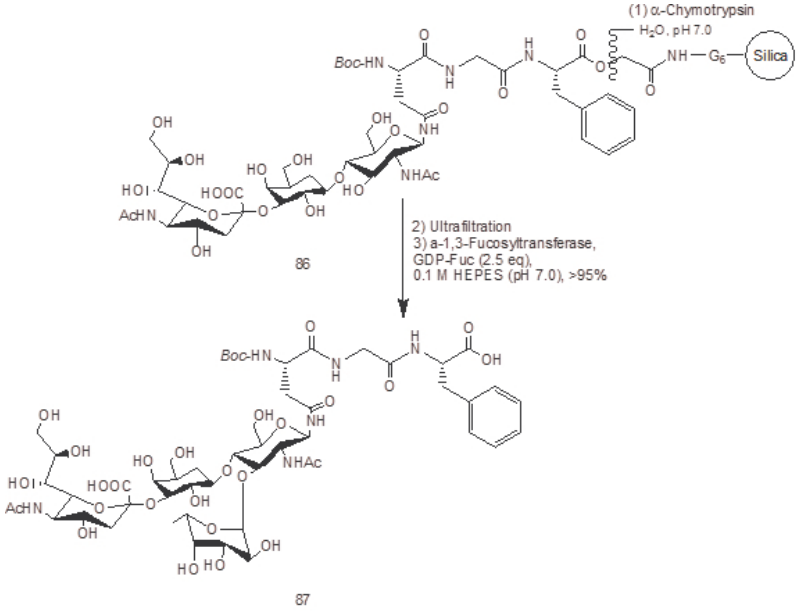


Figure 19. Chymotrypsin catalyzed release of glycopeptides from the solid support [87].

4.2.6. Amylases

One of the most important thermo stable biocatalysts in bioscience, especially in the starch- and food- industry is amylases. Several of main applications are in different fields of development. In the food related industries, amino acids are synthesized by these enzymes. In the chemical, petroleum, and pulp and paper industries, for instance, amylases have been applied for the removal of sulphur containing contaminants via biodegradation of molecules such as dibenzothiophene, in the synthesis of 1,3-propanediol from glycerol and in displacing hazardous chemicals causing toxic yields [3, 88].

There are many researches on ameliorating the selectivity of product or enhancing the yield of cyclodextrin synthesis by cyclodextrin glucanotransferase (CGTase) in the organic media. Furthermore, the transglycosylation of water-insoluble flavonoids are done by CGTase in the organic systems. Sometimes, CGTases are inactivated by highly polar organic solvents. As a result, organic solvent tolerant CGTases are useful for these approaches. *Paenibacillus illinoinensis* strain ST-12K secreted CGTase in a medium overlaid with *n*-hexane. β-cyclodextrin is produced mainly from starch by the purified enzyme. By the addition of ethanol, the total yield of α-, β-, and γ-cyclodextrins was increased 1.4-fold. In particular, in the presence of 10% (v/v) ethanol, the yield of β-cyclodextrin was 1.6-fold that without ethanol. Moreover, addition of ethanol increased the selectivity for β-cyclodextrin product. The CGTases were active and stable in the presence of large scale of different organic media like chloroform, benzene, toluene, 2-propanol, ethanol, and methanol. This is the first research on the organic solvent-tolerant amylase [88].

4.2.7. Epoxide hydrolases

Epoxide hydrolase (EHS) is co-factor independent biocatalyst and ubiquitous in environment. The region- and enantio-selective addition of

water molecules to oxirane rings of epoxides is catalyzed by epoxide hydrolases, resulting in the less reactive epoxide enantiomer and the corresponding optically active vicinal diol. Recently, a number of researches have been done on application of microbial EHS due to their significantly activity in the kinetic resolution of various produced racemic mixtures of different epoxides. Furthermore, many synthetic scale hydrolytic kinetic resolutions of these chiral mediators demonstrated the utility of EHS in organic reactions for producing enantiopure bioactive molecules like ibuprofen and eliprodil [89].

4.2.8. Cutinases

Cutinases have molecular weights of about 20kDa and are the smallest groups of the serine α/β hydrolase subgroup. The hydrolysis of ester-bond of cutin in plants has been catalyzed by cutinases. They also applied to produce agrochemicals including chiral moleculs [76].

Using cutinase for many applications presents an attracting choice over the classical lipases, for example, interfacial activation in not required for these enzymes. Furthermore, cutinases may depict irrespectively activities over reactanst of its being in soluble states or highly retracted, which are otherwise typical behaviors of esterase and lipase, respectively, which causes cutinase ablility to hydrolyzes soluble various esters and emulsifies triacylglycerols. These biocatalysts have favorable activity and stability under reaction conditions, such as presence of organic media, detergents, ionic liquids, oxidizing agents, and proteases, and their activities in esterification, hydrolysis, and transesterification have been noted in recent years [90].

4.2.9. Serine endopeptidases

These biocatalysts are a subfamily of hydrolases that can catalyze the hydrolysis of proteins and peptidic bonds. Endopeptidases have high enantio-, chemo-, and regioselectivity, which are facile to control biocatalysts that do not need cofactors, and are very active under

nonhazardous mild conditions (like pH = 6 – 8). Based on the type of functional group on the active site, it is divided aspartic cysteine endopeptidasess, endopeptidasess, metallo-endopeptidasess, and serine endopeptidasess. Among them, the most applied in biotransformations are serine endopeptidases containing a special triple catalytic activities of three amino acids: histidine and serine-aspartic acid, in a samebehaviorto lipase. As these enzyme presents reversed enantioselectivity relative to lipase, both biocatalysts can be noted supplementary enzymes. Traditionally, these enzymes have been used in the alimentary industry (preparation of soil proteins, quality improvement of bread, fabrication of cheese, etc.). They have been employed as catalysts of different transformations, in the chemical industry, including non-natural substrates, as well as lipases, due to their high stereoselectivity these enzymes. They are widely used as catalysts of esterification/ transesterification reactions in kinetic resolution processes and in the synthesis of peptides. Some of the applications of endopeptidases in the preparation of pharmacologically active compounds by using endopeptidases were detailed [8].

4.3. Lyases

The cleavage of C–N, C–O, and C–C, bonds in diverse hydrolysis processes can be catalyzed by these enzymes, usually leaving double bonds which can be issued to more processes. Systematic denomination follows the pattern substrate group-lyase. The hyphensareessential to avoid any distraction, e.g., the term hydrolyases must be employed in lieu of hydrolyases, which have many similar features to hydrolase [18].

4.3.1. Hydroxynitrile lyases

The cleavage of cyanohydrins to hydrogen cyanide and ketones or aldehydes in the previous stage of ‘cyanogenesis’, can be catalyzed by hydroxy nitrile lyases (HNLs), that is utilized by plants via release of deadly chemical HCN to assert themselves against menaces from pests and fungi (Figure 20) [91].

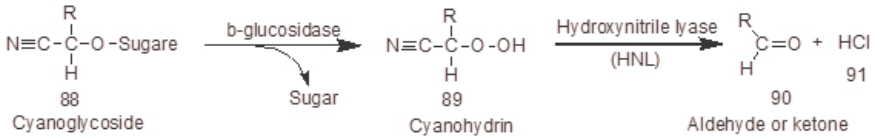


Figure 20. Role of hydroxynitrile lyase in cyanogenesis of higher plants [91].

According to their S- or R-enantioselectivity, HNL is categorized into two distinctive groups of proteins: S-selective HNL including α/β-hydrolase folds and R-selective HNL obtained from oxidoreductase predecessors. The number of characterized R-HNLs is more than those of S-HNLs. Although, there are few researches explained cloning of genes encoding HNL from both groups, there are many reports demonstrated characterization of S-HNL types are extensively scarce than for R types [91].

The stereo-selective formation and cleavage of C–C bond on a widespectrum of α-hydroxy aldehydes and ketones are catalyzed by benzaldehyde lyases obtained from *pseudomonas fluorescens* Biovar I [BAL; E.C. 4.1.2.38]. Almost molecules of BAL are determined by a low solubility in water, i.e., organic media have to be applied to earn related concentrations. This can either be carried out by addition of a water-miscible co-solvent to homogeneous aqueous systems or by presentation of water-immiscible solvents to produce a biphasic solvent in which the enzyme can dissolve in aqueous phase, while reactants are catered and yields are extracted through the solvent phase [92].

4.4. Transferases

These enzymes can shift functional groups from one molecule (or donor) to another one (or acceptor). The principled naming follows the term donor/acceptor group transferase. Generally, the donor groups are coenzymes or cofactors taking activated-chemical groups to be shifted. There are some transferases have been applied in the industrial reasons because of the diverse processes: coupling reactions occur, equilibrium

processes usually do not achieve high scale of products, and the group-transferring molecules are much costly or their products are not recycled easily. However, transferases will be had important roles in the future, if their related challenges are solved. Nevertheless, high stereo- and region selectivity in reactions that are catalyzed by transferases are the main causes for their increasing usefulness. An industrial process applying D-amino acid transaminase (E.C. 2.6.1.21) is shown in Figure 21 [18].

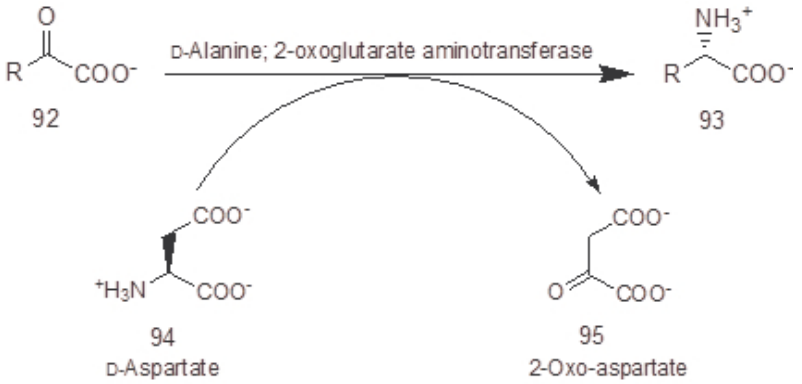


Figure 21. Enzymatic transformation catalyzed by a transferase [18].

4.5. Isomerases

These types are a small group of biocatalysts can catalyze structural and geometric converts within a single molecule and make it suitable to apply cheaper molecules to obtain high-value products. Isomerases can be classified as tautomerases, mutases, trans-cis isomerases, epimerases or racemases, based on the type of isomerization aims. Among them, racemase is more important significantly in kinetic resolutions. Certainly, the most common candidate of these groups is glucose isomerases (E.C. 5.3.1.5), whose some industrial applications are illustrated in Figure 22 [18].

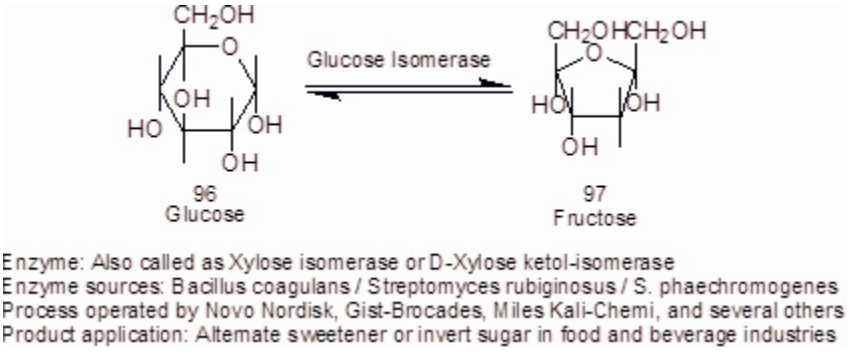


Figure 22. Enzymatic transformation catalyzed by an isomerase [18].

4.6. Ligases

The connection between the two substrates can be catalyzed by these enzymes conjugated with hydrolysis of a pyrophosphate or r triphosphate bond in ATP. There are no industrial processes are reported using ligases at a large amount, but they have a notable role in repairing DNA fragments and in nature, for example, in synthesis of ribosomal peptides or in genetic engineering (like DNA ligases to catalyze formation of C–O bonds in DNA synthesis). The formed bonds are C–N, C–S, and C–O. The systematic naming is follows as X: Y ligases [18].

4.7. Novel enzymes

A demand for novel biocatalysts has been increased by modern industry, which has arisen the progress of new approaches to distinguish biocatalyst-encoding genes. Biocatalysts are highly suitable catalysts for development of eco-friendly industrial studies. Other method to conventional microbial screening approaches is metagenomics. Numerous enzymes with biotechnological importance have been known by metagenomic methods, such as epoxide hydrolase, lipase, amylase, nitrile hydratases, and 2,5-diketo-D-gluconic acid reductase. Synthesis reactions catalyzed by novel enzyme, demonstrate promise to become an important part of such bio refinery processes [93, 94].

In comparison to chemical synthesis methods, enzymatic biosynthesis is non-toxic and non-hazardous. Different combinations of enzymes have generated novel products, often from different systems or organisms, and by using different starting materials for a given biosynthetic pathway. In the future, we definitely will see complex biosynthesis expand into new areas, enabling the food and pharmaceutical industries to produce new dream products and finding environmental and geological applications. Using new interdisciplinary fields pave the path for more studies, and in many approaches, that conventional approaches are still considered as the only method to prepare desired molecules; we will observe the appearance of novel, safer enzymatic processes. It is also expected, enhancing knowledge of metabolic networks, system level biology, and protein-protein interactions to help radical converts in the design of novel usages [82]. According to industrial requirements, the development or discovery of novel enzymatic processes stages can focus on defeating synthetic pinch reactions and improving the operation of traditional chemical processes. Biocatalytic versions of reactions produce high attention and stimulate further studies and reactions progresses in various industries [95].

4.7.1. Combinat enzymes

A myriad of biocatalytic reactions are available for combinatorial biocatalysis. An important issue regarding combinatorial biocatalysis is that of orthogonality. These reactions are ordered in three main categories: (i) modification of existing functionalities; (ii) introduction of new functional groups; and (iii) addition onto functional groups. There are some advantages of enzyme reported to modify existing lead molecules, in comparison chemical reactions: (i) the stereo-control of enzymatic reactions allows a combinatorial approaches to the 3D structure of the molecules, for example, applying DHAP-dependent aldolases; (ii) the regioselectivity enzymes supply the possibility for special mixed modification of lead compounds with multi-copies of same

functional groups, such as glycosylation catalyzed by glycosyltransferases with various regioselectivity; and (iii) high chemoselectivity of enzymatic mechanisms allow modifying only one type of these functional groups in a trellis molecules which involves diverse functional groups [96].

Substrate modifying by a typical enzyme, 'A', prevents it from being substrate for other one, 'B', if the initial substrate modification by enzyme 'B', do not preclude it from being a substrate for the first one, 'A' (Figure 23). The processes are frequently operated. Another round of biocatalytic reactions can modify a first generation of derivatives at additional reactive sites to generate a second production of different derivatives. There is possibility to create numerous derivatives from the original lead molecules after several iterations (Figure 24) [97].

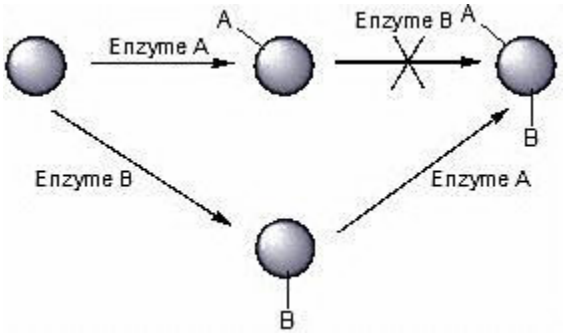


Figure 23. Orthogonality of biocatalysts [97].

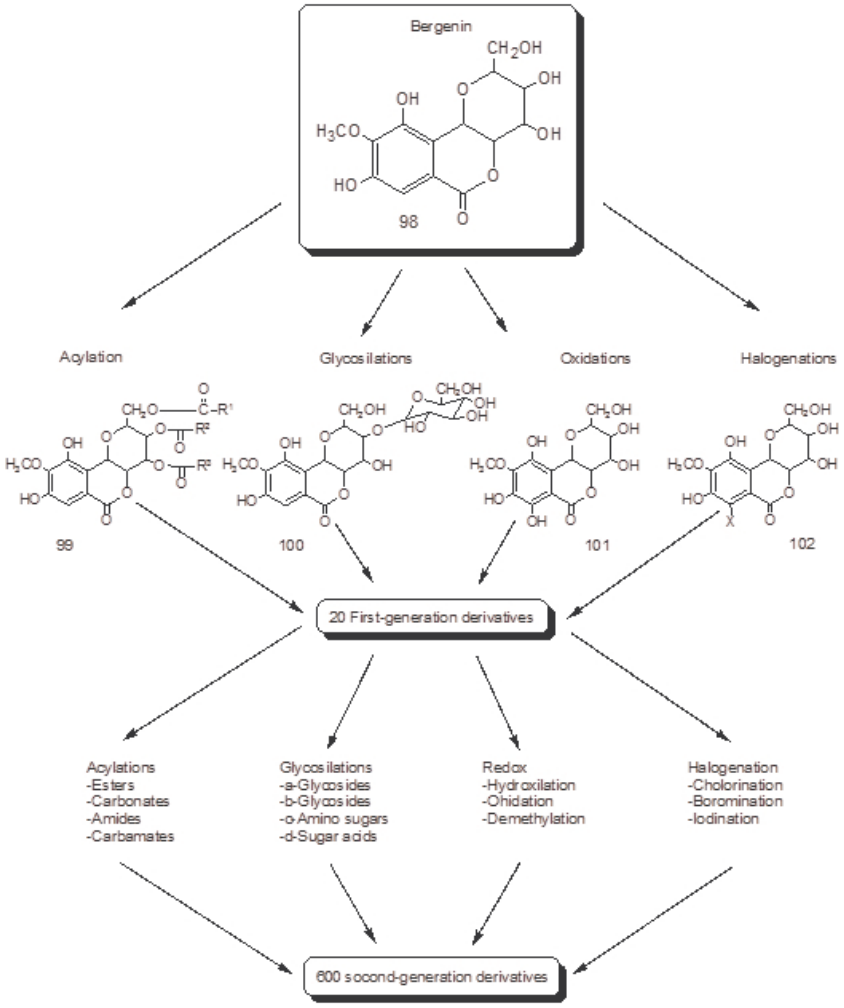


Figure 24. Iterative synthesis of a 600-member library from the flavonoid bergenin [97].

Combinatorial enzymes and engineering process have been explained as useful methods to generate natural products and many of their analogs [82]. High degrees of chemical and structural diversity on initial lead complexes are generated by combinatorial chemistry to optimize them for special targets, bioavailability, binding, etc. Biocatalysts have been recently unified to the group of synthetic methods for combinatorial

organic chemistry with affirmation on focuses libraries. Two different combinatorial figures can be employed by biocatalysts to generate libraries of compounds: (i) combinatorial biosynthesis, that modified or shuffled the genes of natural bioorganic methods to prepare *in vivo* libraries of 'unnatural' natural yields and (ii) combinatorial biocatalysts, that gather *in vitro* new molecules and derivatives by using diverse isolated enzymes [96].

Combination of enzymes or microorganisms a derived from them can synthesize the novel compounds. Various approaches, including the mixed use of more types of microorganism, feeding with an uncommon precursor, or using genetically changed microorganisms (e.g., via the introduction of new genes or by site-directed or random mutagenesis) have been employed. One of the novel structures synthesized by this method is 1-dethia-3-aza-1-carba-2-oxacephem, which includes a mixture of chemical and enzymatic reactions: enzymatic methyl group removal provides a precursor for pursuant chemical preparation of the nonhazardous molecules. Polymers with favorable features obtained from non-costly carbon sources have been produced by recombinant micro-organisms with engineered approaches. Recombinant *Ralstonia eutropha* capable of producing the poly-(3-hydroxybutyrate-co-hydroxyhexanoate) copolymer [P(3HB-co-3HHx)] from fructose was engineered to contain the genes for crotonyl-CoA reductase (CCR) from *Streptomyces cinnamonensis* (ccrSc) and for polyhydroxyalkanoate (PHA) synthase and I-specific enoyl-CoA hydratase from *Aeromonas caviae* (phaC-Jac) [82].

4.7.2. Enzyme-enzyme

The catalyzed hydrolysis by lipase/esterase is carried out in aqueous solvent and can easily be reversible in non-aqueous systems, resulting in transesterification or synthesis of esters. Thus, the combination of lipases/esterases is one of powerful classes of biocatalysts for synthesizing of various organic molecules. Lipase/esterase-catalyzed ring-opening polymerizations (ROP) have been exhibited as a useful process to

synthesize polythioesters, polycarbonates, polyphosphates, and metal-free aliphatic polyesters, under noble reaction conditions. One benign features of this path is that it is eco-friendly, and therefore, could potentially use instead of current toxic catalysts to produce many valuable materials for various applications, such as drug delivery applications, pharmacological, and biomedical [76].

Catalyzed ring-opening polymerizations by lipase/esterase are operated in organic mediums, especially in hydrophobic systems, like heptane, diisopropyl ether or toluene. Due to preserved water layer on the surface of enzyme structures, using hydrophobic solvents results in high monomer changing and Mn (the average of molecular weights) values probably, and therefore, the enzymes catalytic structure will not interrupted. In addition, the green solvents, such as ionic liquids, water, hydrofluorocarbon solvents (HFCs), and supercritical carbon dioxide ($scCO_2$) can also be employed as the reaction medium [76].

Nowadays, lipases/esterases catalyzed production of esters in organic solvent changes to common synthesis technique, because of the possibility of immobilizing such enzymes to improve their stability. There are activated esters that used as acylants in interesterifications to dislocate the reaction equilibrium in favor of ester production. However, using synthetic reactants improve products to be regarded as “natural”; thus, employing different natural alcohols and carboxylic acids accessible on the market causes that the direct esterification be more interesting [98].

5. Enzymes and Dendrimers

Dendrimers are regular tree-like molecules with a globular or disk-shaped structure that offers a unique opportunity to design artificial enzymes and causes macromolecular features, such as cooperativity effects between surface groups and a controlled microenvironment around the dendritic core. Although, the dendrimer synthesizes are difficult and costly processes with an abstruse mixture of yields, but

dendrimers have widely been applied in modern drug delivery sciences, because of high capacity in drug carrying and available multivalent surfaces. The dendrimer concept has exhibited prosperous at delivering applicable macromolecules in useful values from several building blocks and coupling chemistries. They are employed in wide spectrum of medicine and biology to polymers and artificial catalysis. Catalytic structures like metal complexes and cofactors can be located at the core of dendrimers to enable selectivity and microenvironment roles of the dendritic shell. Alternatively, catalytic structures attach in multiple copies at the end of the dendritic branches and create the observed effects. The discovery of dendritic structural space can lead to exploration of applicable dendrimers with properties similar to enzyme, in a process mimicking natural selection. These paths focused on modulating the efficiency and selectivity of catalytic compounds by locating them either in multiple copies at the surface of dendrimer or as a single copy at the dendrimer core [28, 31].

5.1. Dendrimers with a catalytic site at the core

Many researches on dendrimers as enzyme-like biocatalysts have been done on the combination of dendritic branches with catalytic functional groups at the dendrimer core to modify their selectivity. A structure so-called ‘dendrizyme’ have been prepared by an achiral metal chelate surrounded with growing chiral dendritic branches around to catalyze the cyclopropanation of styrene 103 with ethyl diazoacetate 104 to form the cyclopropane carboxylates 105-108 (Figure 25). Ligand 109, with two chiral branches, gave products with 50% enantiomeric excess (ee) (105, 106) and 44% ee (107, 108) without stereoselectivity, but the more ‘dendritic’ complex 110 gave no more than 11% ee [28].

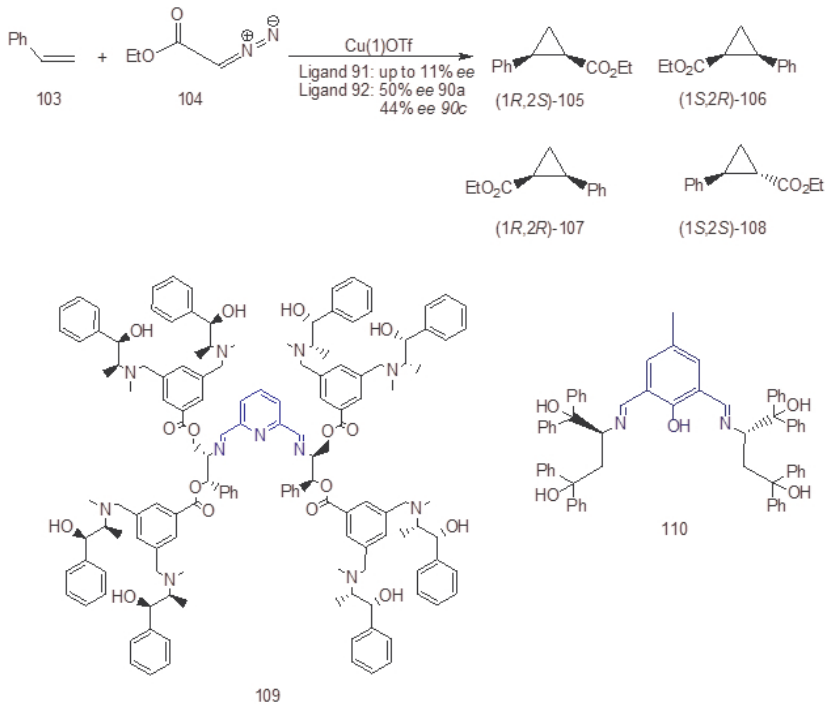


Figure 25. Brunner's dendrizyme ligands reported in 1994, active for the cyclopropanation of styrene [28].

Microenvironment can be obtained by dendritic shells to prepare shielding for all functional groups at the dendrimer core or create favorable catalysis. This principle in the catalyzed elimination of HI from an iodoalkane to create an alkene has been demonstrated by a dendrimer possessing a polar core. The elimination process does not occur in the surrounding hydro-carbon solvent, but happens in the polar interior dendrimer. In a same work, the tertiary alcohol linalool esterification was catalyzed by a polar dendrimer interior with three nucleophilic 4-(pyrrolidino) pyridine compounds at the core with triethylamine and pivalic anhydride in cyclohexane medium, under which conditions the non-dendritic catalysts 4-(pyrrolidino) pyridine has less activity [28].

5.2. Dendrimers with multiple catalytic groups

The multivalent display of functional groups at the end of dendritic branches is a characteristic feature of dendrimers. Poly (propyleneimine) (PPI) and PAMAM are comercially available with 4, 8, 16, 32, and 64 amino groups. Such multivalence is also realized in dendrimers based on 3, 5-dihydroxy-benzylalcohol, lysine, 2, 2-bis (hydroxymethyl)-propionic acid, [tris (2-ethoxycarbonyl ethoxymethyl) aminomethane], P = N–P = S linkages and glycerol (Figure 26) [28].

Dendronized polymer versions of the system were as active as the dendrimer catalyst, suggesting a microenvironment effect. In a converse example, the hydrophobic core of dendrimer 114, equipped with a benzophenone photosensitizer, could catalyze the [4 + 2] cycloaddition of singlet oxygen to cyclopentadiene 111, with methanol as a polar solvent (Figure 26). The hydrophobic dendritic microenvironment allowed binding of the non-polar cyclopentadiene substrate 6, but not the more polar unstable cycloaddition product 112. The diol 113 formed *in situ* by reduction of 112 with thiourea. Up to 40% conversion of cyclopentadiene 111 was observed in the presence of 0.1mol% of the dendritic benzophenone 114, whereas less than 10% product was formed with the same amount of non-dendritic benzophenone activator [28].

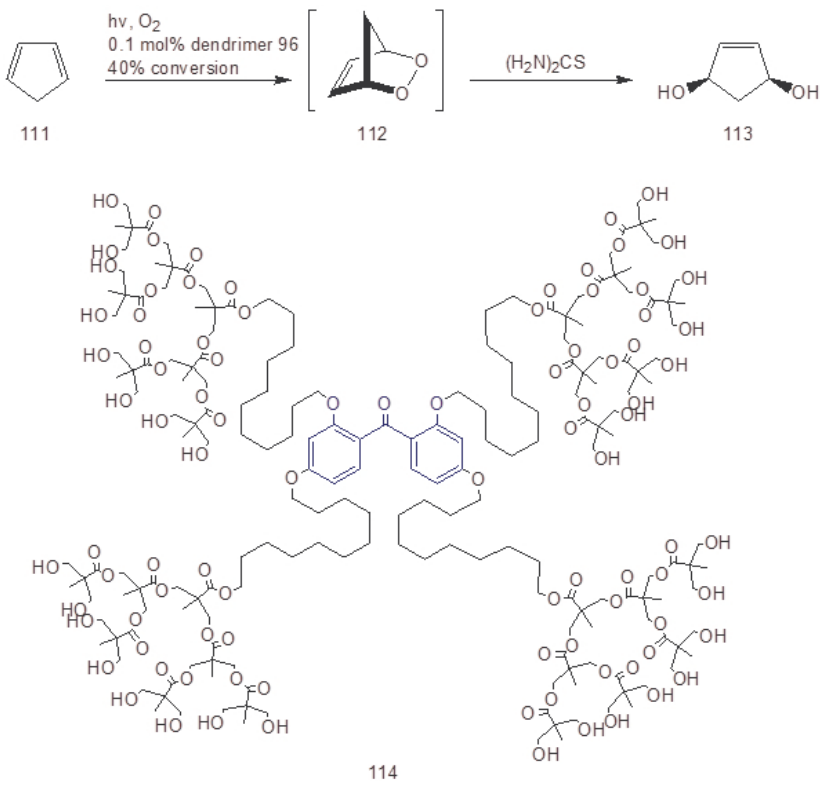


Figure 26. Singlet oxygen is generated in the benzophone core of dendrimer 114 and reacts subsequently with 111 in a [4 + 2] cycloaddition reaction to form 112, which is reduced *in situ* to form diol 113 [28].

The dendritic multivalent display of catalytic groups has been studied by nanofiltration or precipitation to create macromolecular catalysts that are easily separable from products. Although, a few reports indicate that dendritic multivalent display of catalysts sometimes results in higher catalytic performance, this technical advantage is very useful independently of any rate or selectivity effect of the dendrimer structure on the catalyst. There is reported the PAMAM dendrimer 118 with four Co-Salen complexes. The hydrolytic kinetic resolution of (rac)-1,2-epoxycyclohexane 115 is catalyzed by this dendrimer with 24-fold higher reactivity per Co-Salen unit and higher enantioselectivity (99.2% ee of diol product 117 at 43% conv.) compared with the metal complex alone

(98.2% ee at 29% conv.), indicating a positive cooperative effect (Figure 27). Unfortunately, the catalytic group reactivity of dendritic multivalent also often reduced. For example, PPI-dendrimers functionalized with imidazoles for the hydrolysis 2,4-dinitrophenyl acetate in water, and with phosphazene bases for base-promoted carbon-carbon bond forming reactions such as Michael and Nitroaldol additions [28].

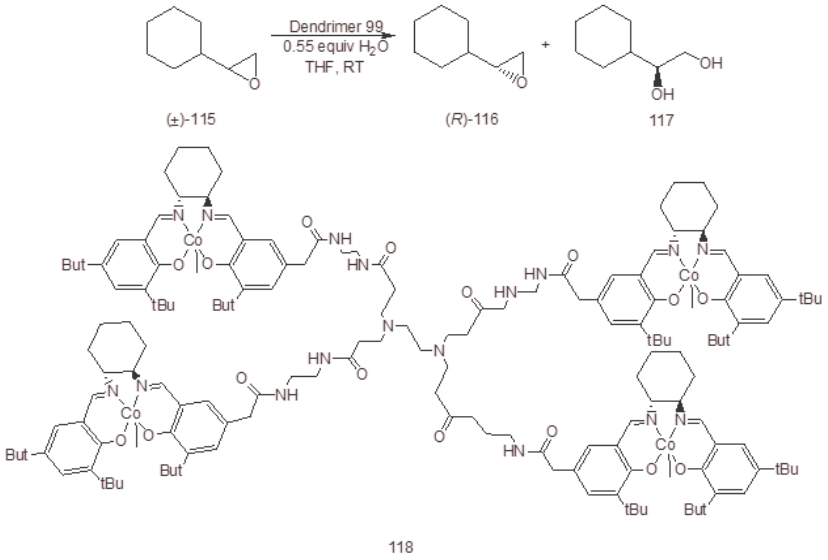


Figure 27. The dendritic version 12 of Jacobsen's epoxide hydrolysis catalyst shows cooperativity (25-fold higher reactivity per group) and comparable enantioselectivity for the hydrolysis sis of racemic epoxide 10 (99.2% ee (enantiomeric excess) of diol product 11 at 43% conv.) [28].

5.3. Catalytic peptide dendrimers

Artificial enzymes can be created by using peptide dendrimers enforced globular shape of dendritic peptides, and capitalizing on the simplified synthesis. Almost difficult problem of protein folding are circumvented by this strategy, and should render peptide dendrimers more biodegradable than natural proteins and relatively more stable towards denaturing conditions (high temperature or non-aqueous conditions). The executed globular shapes are investigated to show

general intramolecular interactions between various amino acids resulting macromolecular function than intermolecular interactions leading to precipitation and aggregation, as is usually found with linear peptides. The displays of ligands have been multivalent using peptide dendrimers as diagnostic reagents, vaccines and drug, anticancer and antiviral agents, protein mimetics, and gene delivery vehicles. HIV infections can be prevented by multivalent aryl sulphonate derivatives as polylysine dendrimers, which equipped with different end groups being appraised as drugs [28].

Recently, functional selection and the basic progressive stages of various productions have been shown for peptide dendrimers based on combinatorial chemistries. Eight variable amino acid positions prepared split-&-mix combinatorial libraries of peptide dendrimers befalling as a couple in four consecutive branches of third peptide dendrimer production (Figure 28). Sixteen diverse amino acids were applied and locating four different amino acids at each location to prepare a library of 4^8 different dendrimers. Direct on-bead screening for hydrolysis of the fluorogenic butyrate ester 119a generated some fluorescent beads that were chosen by sequenced, isolated, and inspection. The peptide dendrimer was reproduced and purified, and obtained peptide dendrimers demonstrated the desired esterase-like activity with the pyrene trisulphonate ester 16a and its nonanoate and acetate derivatives(119b and 119c), affirming the combinatorial approaches [28].

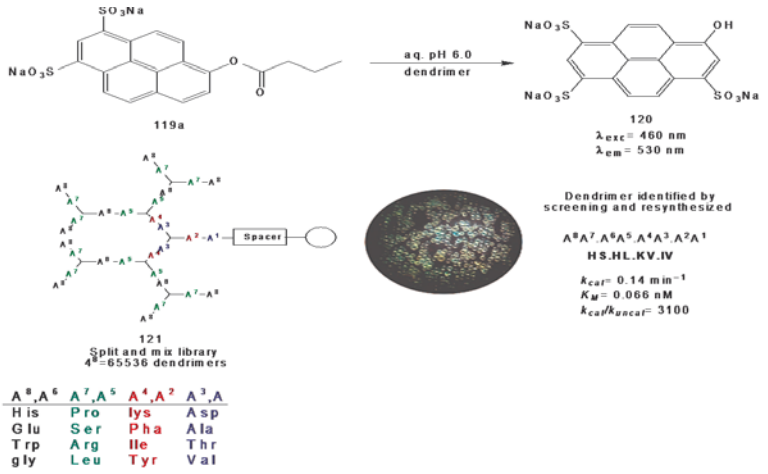


Figure 28. Combinatorial library of peptide dendrimers [28].

6. Whole Cells Catalyze

In recent decade, enzymatic processes that use isolated enzymes or whole cells as biocatalytic reagents, have found increasingly widespread usages [99]. Using whole microbial cells as biocatalysts is smarter than isolated biocatalysts. This is chiefly the case for different redox bio reactions, where the enzyme is not stable outside the cell and/or cofactor regeneration is mandatory. In this aspect, the internal cellular metabolisms cause the reproduction of cofactors. Herein, oxidoreductases are applied as whole cells. They are also employed, while multi-step processes are forecasting, given the presence of a multi-enzyme cluster, among the cellular reactions for co-factor reproduction. Whole cells can catalyze some bio reductions to produce chiral building blocks (Figure 29). However, using whole cells as sacs of biocatalysts in bioconversions have several difficulties: (i) the reactants or yields degradation occur cellular metabolism; (ii) prohibition for cell growth by the reactants or the yields; (iii) limited permeability of the substrate or the product; and (iv) low efficiency, weak enzyme activity, and the work-up step of reaction very complicated. In any case, if whole cells applied, the process could more economically attractive than isolated enzymes. Thus, using whole cells forms 75% of the industrially implemented redox biotransformations [8].

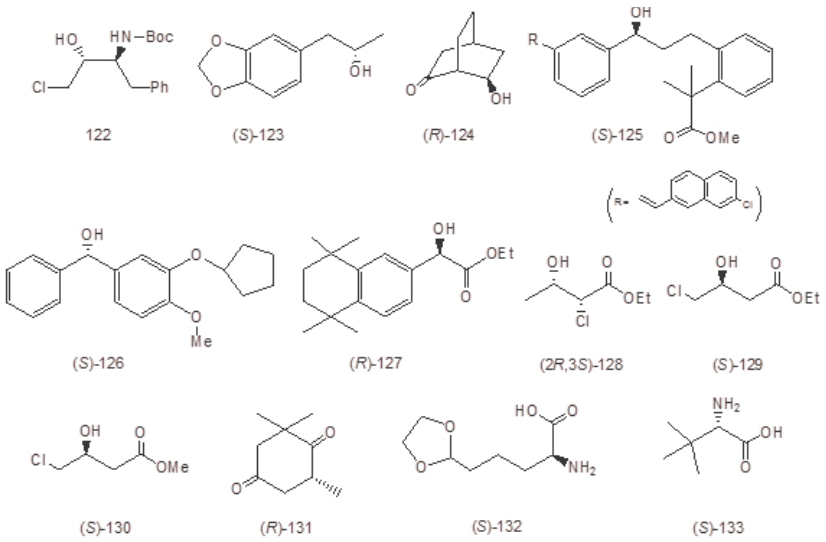
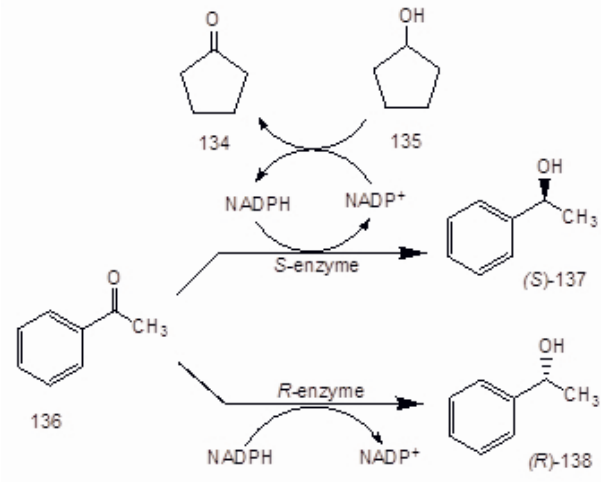


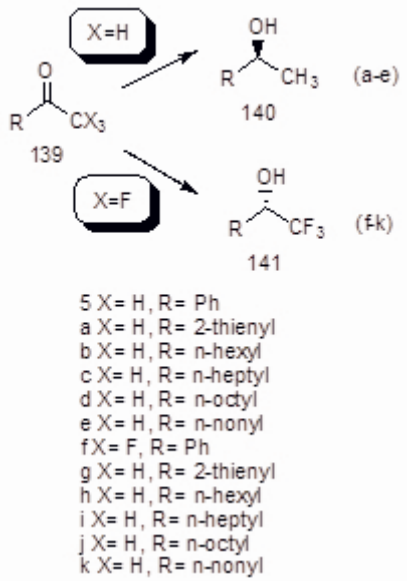
Figure 29. Some chiral building blocks obtained through bioreductions catalyzed by whole cells [8].

Das-Bradoo et al. [100] showed that the obtained whole cells from alkaliphilic *Bacillus pseudofirmus* AR-199, induced for β-galactosidase activities, can be employed for preparation of 1-octyl-β-d-galactoside or 1-hexyl-β-d-galactoside, by transglycosylation process between alcohol acceptor and lactose.

When cyclopentanol was used as the sacrificial substrate to regenerate NADPH, a prepared acetone powder from dimorphic fungi *Geotrichum candidum* IFO 4597 produced high enantioselectivity. The existence of R- and S-dehydrogenases in *G. candidum* explained these phenomena (Figure 30(a)). These dehydrogenases used NADPH but cyclopentanol could oxidize by the former. Apparently, once produced in the active site of S-enzymes, separation of NADPHs were slow enough which the R-enzymeswereincapable to disposal the co-factor and thus added ketones were not reduced. Reduction of substituted acetophenones with high stereoselectivities was carried out by the acetone powder of *G. candidum* with co-factor regeneration by cyclopentanol (Figure 30(b)) [101].



(a)



(b)

Figure 30. Reductions catalyzed by *G. candidum* dehydrogenases: (a) two reductases (R- and S-enzymes) are present; (b) two enzymes show opposite stereoselectivities for reducing non-fluorinated versus fluorinated ketones [101].

7. Mesophilic, Thermophilic, and Thermostable Enzymes

These biocatalysts are active and stable at those temperatures that are very high compared to the optimum temperatures for the microorganisms growth. These enzymes are generally isolated from thermophilic organisms and have known numerous industrial applications due to their generalintrinsic stability. Among the applications of thermostable biocatalysts, the most extensively applied oneisamylase in the starch industries. In the food industries, the amino acids have been synthesized by them. In the pulp and paper, chemical and petroleum, industries, they have the elimination of sulphur containing pollutants is possible by using these enzymes through the biodegradation of compounds like dibenzothiophene, in the production of 1,3-propanediol from glycerol and in replacing polluting chemical reagents causing toxic products. It has been shown extremophilic marchaea, fungi, and bacteria will colonize environment was believed to be impossible to survive in. In fact, their diversity has not been successfully explored yet. These enzymes can be isolated from organisms have just commenced providing changes under conditions that are suitable for industrial approaches. These thermostable enzymes need mild and environmentally benign conditions required for some specific reactions that are not possible by chemical catalysts, as compared to the temperatures and pressures required for chemical conversions. As the availability of thermostable enzymes is becoming more available several new applications in the future is probable. High production of the enzymes for the industries still is a challenge, although it is believed that they provide tremendous economic benefits [3].

7.1. General advantages of thermophilic enzymes

As compared to traditional chemical processes, the higher specificity and the reduced process temperatures in biocatalytic methods, exhibit more important benefits in production of multifunctional or thermolabile acids/esters: (i) less by-products; (ii) facile waste stream therapy, owing to absence of acidic solvents and catalysts; and (iii) lower energy consumption [102].

Because of this fact that thermostable biocatalysts are better investigated for rough industrial processes, they have gained industrial and biotechnological interest. Several industrial usages and biocatalytic transformation of these enzymes are exhibited in Table 6 [3].

Table 6. Bioconversion reactions and applications of thermostable enzymes [3]

Enzyme	Temperature range (°C)	Bioconversions	Applications
α-Amylase (bacterial)	90-100	Starch ⟶ dextrose syrups	Starch hydrolysis, brewing, baking, detergents
α-Amylase (fungal)	50-60	Starch ⟶ dextrose syrups	Production of maltose
Pullulanase	50-60	Starch ⟶ dextrose syrups	Production of glucose syrups
Xylanase	45-65, 105[a]	Craft pulp ⟶ xylan + lignin	Pulp and paper industry
Chitinase	65-75[b]	Chitin ⟶ chitobiose Chitin ⟶ N-acetyl glucosamine (chitibiase) N-acetyl glucosamine ⟶ glucosamine (deacetylation) Chitin ⟶ chitosan (deacetylase)	Food, cosmetics, pharmaceuticals, agrochemicals
Cellulase	45-55, 95[c]	cellulose ⟶ glucose	Cellulose hydrolysis, polymer degradation in detergents
Protease	65-85	Protein ⟶ amino acids and peptides	Baking, brewing, detergent, leather industry
Lipase	30-70	Fat removal, hydrolysis, interesterification, alcoholysis, amynolysis	Dairy, oleo chemical, detergent, pulp, pharmaceuticals, cosmetics and leather industry
DNA polymerase	90-95	DNA amplification	Genetic engineering/PCR

[a]Xylanase from *Thermotoga* sp.

[b]Within this range enzyme activity was high.

[c]Cellulase from *Thermotoga* sp.

Micro-organisms can be adapted to the condition they have to live and survive. Thermophiles with protein structure are thermostable and demonstrate resistance against denaturation and proteolysis. After thermophiles denaturation to refold the proteins to their native form and restore their functions, these organisms are used to produce specialized proteins known as chaperonins. Saturated fatty acids build the cell membrane of thermophiles. Fatty acidscan prepare a hydrophobic environment for cells and cause the cell is rigid to live at high temperature. The archaea, which build the majority of the hyperthermophiles, have lipids jointed with ethers on cell wall. These layershavehigh resist against heat even more than membranes produced from fatty acids [3].

7.2. Thermostable lipases

Candida antarctica lipases are efficient biocatalyst for a lot of organic reactions, such as stereo-selective transformation synthesizes. Industrial processes happen at very high temperatures owing to the advantages of these conditions, like contamination risk, decreased viscosity, enhanced solubility, and consequently, faster reaction rate. Thus, desire thermostability of special biocatalyst particularly is more essential in chemical processes. Due to the inactivation of CalB, the conversions for glycerol carbonate from glycerol were cut down over 60^{o}C by this enzyme. Moreover, thermostable CalB is needed to prepare glycerol carbonate due to two molecules (dimethyl carbonate and glycerol) can be dissolved completely each other around over 70^{o}C. Random processes obtained only marginal prosper in upgrading thermostability of CalB, although these approaches have been applied for increasing the thermostability of CalB [103].

Nowadays, many researches are significantly reported about lipase-catalyzed acylation reactions, which up to recent decade have not been studied very so [104].

7.3. Thermostable xylanases

Xylans are a large group of the most plentiful organic substances in the nature and have wide applications in those industries like pulp and paper. Using xylanases at high temperatures may disturb the cell wall compounds. This, as a result, facilitates lignin removal in different steps of bleaching, since it is attached to hemicellulose, especially xylan, in cell wall. Xylanases for such a purpose (i) require being of low molecular weights to help their propagation in the pulp fibers; (ii) the lack of cellulytic activities is vital to eschew hydrolysis of the cellulose fibers; and (iii) high product of biocatalysts should be observed at an inexpensive condition. There is reported the optimum temperature for xylanase activities is 50-60ºC with a half-life of around 1h at 55ºC. It have also been reported that some xylanases show higher thermal resistance and optimal activities in the range of 80ºC to 100ºC [3].

7.4. Thermostable cellulases

Cellulose is much stable against the digestion and hydrolyses due to the difference in the type of bond and the highly ordered crystalline form of the compound between starch and cellulose. Cellulase is a complex enzyme includes exoglucanases, endoglucanases, and β-glucosidases is needed all for the complete hydrolysis of cellulose. Cellulose as an endoglucan, can be randomly hydrolyzed by endoglucanse cellulase and produce oligosacaccharides, cellobiose, and glucose, and as an exoglucan, it can be hydrolyzed by exoglucanase cellulose, which hydrolyzes β-1,4-Dglucosidic bonds in cellobiose that release cellulose from non-reducing end. In other words, hydrolyzing cellobioses to glucoses can be carried out by obtained β-glycosidases from thermophilic origins that have taken renewed interests in pharmaceutical industries [3].

7.5. Thermoactive chitinases

This biopolymer usually found attached to other proteins and polysaccharides. After cellulose, the most abundant natural biopolymer is chitin, which includes a linear β-1,4-homo-polymer of N-acetylglucoseamine tailings. The most important applications of

chitosan are: heavy metal chelating agents, preparation of cosmetics, paper production, textile finishes, wastewater clearing, cements, photographic products, and for medical and veterinary purposes. The main sources of chitin are including shrimp and crab residue, cell walls of fungi, and the insects shell [3].

7.6. Thermostable proteases

Bacilli organisms have obtained the focus of attention in biotechnology. Since, the isolation of Bacilli from diverse sources is relatively facile. A *Bacillus* sp. has synthesized thermostable proteases that havehigh activities at 60°C. Although, some thermophilic *Bacillus* sp. that give various proteases have been isolated, the first isolate one being *Bacillus stearothermophilus* that is stable at 60°C [3].

7.7. Mesophilic organisms

Oil rich soil produce mesophilic organism *Acinetobacter* sp. EH28 that synthesis alkaline thermostable lipases. It was purified by hydrophobic interaction chromatography and ammonium sulphate precipitation around 24.2-fold to generate specific activities of 57.1U/mg (i.e., enzyme U/mg soluble protein). One of recognizable properties is include its stability towards high temperatures and organic medium. The distinguishing specifications and its applicability in organic reactions significantly show EH28 lipases are a group of green catalysts. Purified lipases demonstrated notable esterification activities (63%) for ester ethyl caprylate production, while their efficiency enhanced by 1.43-fold (90%) over the immobilization in MBGs [52].

8. Cross-Link Catalysts

Although biocatalysts are useful tools for a green and sustainable industry, production, and operating costs have limited their synthetic application [105]. Coupling reactions are reactions which two organic substrates (often in the similar size) are joined by a catalyst. These reactions have usually been referred to coupling of small substrates to

give natural or artificial synthetic polymers. Coupling reactions are important in most industrial processes and many researches have been done in this field to improve the enzyme properties. Coupling reactions are divided into two main classes: (i) homocoupling, in which the reactant molecules are the same; and (ii) cross-coupling, in which two different molecules react to form one new molecule [38, 105].

In solution, the original protein structure essentially is maintained by the hydration shell. A hydration shell formed of water molecules surround protein structures in medium which can mainly join to the protein surface by hydrogen bonds. In presence of polar organic solvent is solution, the interactions cause to keep the enzyme molecule in its native conformation are changed because the polar organic molecules tend to displace water from the hydration shell, and thus, may finally unfold the protein. The reason of protein denaturation by organic systems is the demolition of hydration shells. Increasing the severe reduction of the cosolvent content in the instant adjacency of enzyme compounds is desired approaches for protection of proteins from some denaturations. Herein, there are several methods have been reported like: proteins entrapment by hydrophilic matrixes, cross-linked enzyme aggregates (CLEA), cross-linked enzyme crystals (CLEC), and reverse micelles [106].

Sometimes, the thermo-stabilization of enzymes is weakly occurred by hydrophilization, thus the cross-linking of enzymes by the polymer has only a marginal effect on the rigidity of the enzymes. If the stiffness of biocatalysts had practically been increased, an increasing in thermal stability of them has been obtained. Therefore, it seems the hydrophilization be effective in hampering the unfolding of hydrophobic cores that happen when the protein is depicted to low polar medium in the existence of co-solvents [106].

8.1. Cross-linked enzyme crystals (CLEC)

The most frequent applications of cross-linked enzyme crystals have been found in biotransformations. The influence of organic solvents on enzymatic enantioselectivity was studied by using CLC-subtilisin. Theoretical models were developed and discussed to predict these solvent effects. The experimental results demonstrated comparable enantioselectivity of CLC-subtilisin and lyophilised in several organic solvents. In the hydrolysis of peptide amides, amino acids, and enantioselective hydrolysis of chiral esters, the efficiency of CLC-subtilisin in organic production of peptidomimetics or different peptides was studied in detail. Stabilizing the enzymes and modifying their catalytic activity were carried out by the chemical modification of enzymes. In semi-synthetic biocatalystsan active group as a catalytic reagent is chemically induced into the peptide structure of initial biocatalyst and the main enzymatic activities are completely converted. Many features of these enzymes such as substrate dependence or enantioselectivity can be studied based on this template by using a well-known enzyme template. The concepts of CLCs and semi-synthetic biocatalysts were mixed, resulting in a novel kind of designed enzymes. Figure 31 shows the chemically transformation of subtilisin into the cross-linked enzyme crystals of semi-synthetic peroxidase seleno-subtilisin [107].

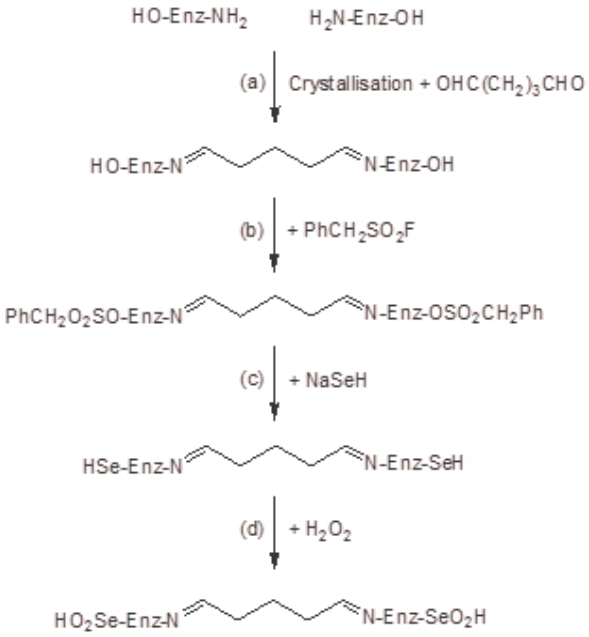


Figure 31. The preparation of CLCs of the semi-synthetic peroxidase seleno-subtilisin (a) batch crystallisation in 12% (w/v) Na_2SO_4 at 16-18ºC was followed by treatment (45 min) with glutardialdehyde at pH 7.5; (b) 1h in 0.11M phenylmethanesulphonyl fluoride at pH 7.0; (c) addition of aqueous sodium hydrogen selenide solution was followed by 45h stirring at 45ºC; and (d) 30 min in 10mM H_2O_2. Enz, enzyme; Enz-OH, Ser221 of subtilisin; Enz-NH2, lysine residues on the surface of the enzyme; Ph, phenyl [107].

Resulted CLC-seleno-subtilisins can blend the features of chemical engineering aspects and their catalytic activities are changed from proteases to peroxidases by biocatalyst. The CLC-seleno-subtilisin can catalyze the reduction of racemic hydroperoxides with desired enantioselectivities corresponding to the enantioselective reactions of subtilisin. On the other hand, since the stability was tested at 40ºC in 50% acetone or DMF or at 60ºC in 100% aqueous buffer, the typical properties of cross-linked enzyme crystals were observed. This type of cross-linked enzymes can recycled around ten times with any selectivity

or activity reduction, a unique feature of these enzymes; additionally the high stability of them under these conditions was similar to those of CLC-subtilisins over 10 days [107].

The properties of cross-linked enzyme crystalline structure have gained attention as well as the catalytic activity of them. The crystalline materials are highly porous in compare with zeolites, which consist of an aluminium silica framework. The aperture diameter and particle sizedepend on the type, pore surface area, and the preparation mode of proteins. In comparison to inorganic zeolites, protein structure is chiral and it has special groups for binding to diverse molecules [107].

CLCs are employed in three fields. First, using in biotransformation that is the more important usage. These crystals have good stability and can be frequently reused. A novel kind of chemically engineered enzymes has depicted by mixing the cross-linking of enzyme crystals with the chemical modification of their active site. Second, the XRD researches in organic medium are their 'classical' application. This technology helped our knowledge about molecular basis of enzyme operation in organic systems. Third, the newest usage of these crystals is in the chromatography. These protein crystals were investigated, characterized, and applied as stationary phases [107].

8.2. Cross-linked enzyme aggregates (CLEA)

These immobilized enzymes are robust with high activity with controllable particle size from 1 to 100 micrometer. In the 1990s, the first steps in using cross-linked enzyme crystals (CLECs) as industrial biocatalysts were taken by Altus Biologics. It is proved that CLECs are stable against denaturation by high temperature, proteolysis, and organic media compared with freeze-dried (lyophilized) powder or soluble biocatalysts. They are common in industrial biotransformations due to their overall stability and facilitate in recycling, coupled with their high volumetric and catalytic efficiencies. However, they have an intrinsic disadvantage: crystallization of enzymes is a so difficult process requiring high pure enzymes, which deterrently needs high costs. The more recent advanced CLEAs are obtained by physical aggregates of protein molecules such simple sedimentation of biocatalyst from aqueous medium, by the addition of salts, or non-ionic polymers or water miscible

organic systems. Non-covalent bonding can be held the physical aggregates together with no denaturation and disturbance of their tertiary structures. Subsequently, cross-linking of these physical aggregates makes they are insoluble frequently while maintaining their pre-organized superstructure and catalytic activity. Herein, cross-linked enzyme aggregates (CLEAs) as a new group of immobilized enzymes are developed by this novel method. Purifying enzymes is carried out by precipitation from an aqueous medium with addition of polyethylene glycol or ammonium sulphate; the CLEA methodologies essentially merge immobilization and purification into a single unit operation, which do not require a highly pure enzyme. It could be employed for the direct isolation of an enzyme from a crude fermentation broth, in an immobilized and purified form suitable for performing biotransformations [108-110].

Due to CLEAs effective, inexpensive, and facile synthesizing method, they are very attractive biocatalysts. They can also be reused readily and demonstrate better performance and stability. The methodology is applied to bioctalysts especially cofactor dependent redox enzymes. Application to penicillin acylase employed in antibiotic production exhibited many advantages over other biocatalysts. The numerous potential usages of CLEAs are: (i) synthesis of fine chemicals, biofuels, pharmaceuticals, flavours and fragrances, agrochemicals, nutraceuticals, and bulk monomers; (ii) biosensors/diagnostics, e.g., glucose oxidase and cholesterol oxidase biosensors; (iii) delivery of proteins as therapeutic agents or nutritional/digestive supplements, e.g., beta-galactosidase for digestive hydrolysis of lactose in dairy products to alleviate the symptoms of lactose intolerance; (iv) cosmetics, e.g., in skin care products; (v) oils and fats processing, e.g., in bioemulsifiers, biolubricants, and bioemollients; (vi) food and beverage processing, e.g., lipases in cheese manufacture and laccase in wine clarification; (vii) detergents, e.g., proteases, amylases, and lipases for removal of protein, carbohydrate, and fat stains; (viii) waste water treatment, e.g., for removal of phenols, dyes, and endocrine disrupters; (ix) animal feed, e.g., phytase for utilization of organically bound phosphate by pigs and poultry; (x) carbohydrate processing, e.g., laccase in carbohydrate oxidations; and

(xi) pulp and paper, e.g., in pulp bleaching. As a typical example, lipase CLEA showed 12-fold increase in activity over free enzyme powder when the CLEA was used in transesterification of tributyrin [109-112].

9. Cofactors

Cofactors have non-protein structures, can bind to a protein and are required the biological activities of protein. These proteins are usually biocatalysts, and cofactors may be noted "helper molecules" that aid in various biochemical transformations. Many organic cofactors are generally vitamins or made from them. A lot of contain the AMP (nucleotide adenosine monophosphate) as section of their structures, such as ATP, coenzyme A, FAD, and NAD^+. This common structure may reflect a usual evolutionary origin as section of ribozymes in previous RNA science. They have been offered that the AMP part of structure can be noted a type of "handle" by the biocatalyst can "grasp" the coenzyme to substitute them among various catalytic centers [113].

NAD^+ is the first identified cofactor, which A. Harden and W. Youndin 1906 discovered it. They noticed that yeast extract filtering and adding boiled can speed up alcoholic zymosis in unboiled extracts. They named the unrecognized factor responsible for these effects conferment. Through a difficult and complex filtration from yeast extracts, the se thermo stable factors were recognized as nucleotide sugars phosphate by Hans von Euler-Chelpin. During the early 20th century, there were identified other cofactors, with ATP being isolated and purified in 1929 by K. Lohmann, and coenzyme A being found in 1945 by F.A. Lipmann. The applications of these compounds were first lycryptic, but, in 1936, O.H. Warburg discovered the application of NAD^+ in different hydride transfers. This exploration was extended in the early 1940s by H. Kalckar's researches, who established the relation among ATP generation and sugar oxidation. This certified the effect of ATP in the energy transfers which had been of feredin 1941 by F.A. Lipmann. Afterwards, A.L. Lehninger and M. Friedkin in 1949 proved that NAD^+ joined metabolic approaches like the ATP production and the citric acid cycles [114].

They can be categorized based on how strongly attach to enzymes, with coenzymes and prosthetic groups that bound loosely and tightly cofactors, respectively. Some sources can also confine using cofactors to inorganic molecules. Apoenzyme is an inactive biocatalyst with any cofactor and other enzyme with cofactor is called holoenzyme [114]. Cofactors are classified into two main subfamilies: inorganic cofactors include iron-sulphur clusters or metal ions Mn^{2+}, Cu^{+}, Mg^{2+}, and organic cofactors likeheme or flavin. Recent groups can be more divided into prosthetic groups and coenzymes. The concept coenzyme specifically mentions to biocatalysts and the applicable features of proteins. In other words, “prosthetic groups” refer to the nature of binding between protein and cofactor (covalent or tight) and also refer to structural properties. Several sources generate some various compliments of prosthetic groups, cofactors, and coenzymes. There are considers strongly bound organic structures as prosthetic groups but not as coenzyme, while alters introduce all non-protein organic structures required for activity of biocatalysts as coenzymes, and categorize those that are bound powerfully as coenzyme prosthetic groups. It must be mentioned that these types are loosely applied [6]. Organic cofactors are small organic compounds (generally with Mw less than 1000Da) which take part directly in reaction and can be attached either tightly or loosely to biocatalyst [114-116].

In recent one, it may be called a prosthetic group when it is not easy to remove without enzyme denaturation. It is necessary to mentioned that there is no division among tightly and loosely bound cofactors. In fact, some kike NAD^{+} can be strongly bound in many biocatalysts, when it is bound loosely in others. There is thiamine pyrophosphate (TPP) as another example is bound tightly in pyruvate decarboxylases or transketolases, although TPP bound in pyruvate dehydrogenase is less strong. Other coenzymes, for instance, lipoamide, biotin, and FAD (flavin adenine dinucleotide), are covalently bound. In generally, tightly bound cofactor is reproduced during similar cycles, but the other one (loosely bound cofactors) can be reproduced in a subsequent process catalyzed by a alter biocatalyst. In the recent one, the cofactor can also be noted as co substrates or main substrates [114].

Different vitamins can be used as coenzyme (like vitamin C) or as precursors to various synthetic cofactors (like folic acid, vitamins B1, B2, B6, B12, and niacin). However, vitamins have other applications in natural metabolisms. There are organic cofactors containing a nucleotide like electron carriers FAD and NAD, and acyl group carriers such as coenzyme A. The majority of these cofactors are observed in diverse species, and many others are universal to all aspects of our life. There is a group of unique cofactors as an exception to this vastrepartition that revolved in methanogens, which are confined to these types of archaea [117].

Biochemical reactions involve electron transferring from one molecule to another one are catalyzed by a broad range of enzymes, namely, oxidoreductases or redox enzymes (Dixon and Webb, 1979). Oxidoreductases often utilize (in) organic cofactors to perform corresponding reactions (Figure 32) [36].

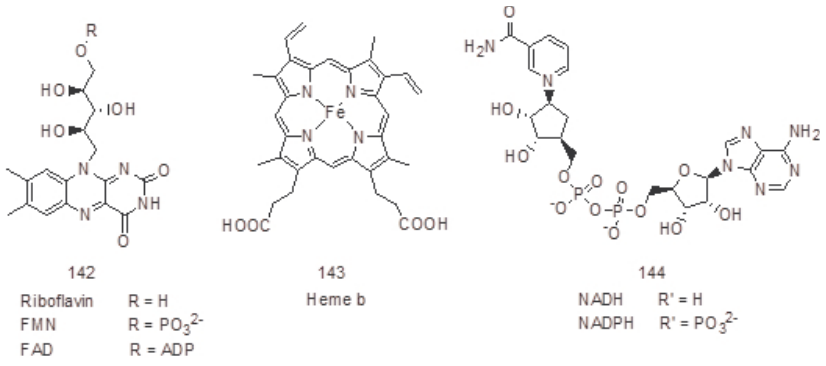


Figure 32. Examples of cofactors utilized by oxidoreductases [36].

Redox enzymes area round 25% of current biocatalysts. Owing to their high specificity, efficiency, and selectivity, they are interesting as catalysts for reduction of environmental contaminants, bio production of polymers and chiral structures, and production of biosensors. While they usually need cofactors like nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD), their applicable usages can be a serioud problem. These cofactors are generally expensive,

thus regeneration of them in the industrial scale biotransformations using oxidoreductases is vital to the economic viability. There have been explored a vast range of redox enzymes applications, including clinical applications, production of chiral molecules like chiral acids, aldehydes, and alcohols; production and modification of polymers, especially biocompatible or biodegradable ones; and degradation of organic pollutants; and biosensors for a variety of analytical [118-120].

For the preparation of enantiomerically pure compounds, enzyme catalyzed processes by means of coenzyme-dependent enzymes are of increasing interest [121]. These enzymes catalyze oxidoreduction reactions; namely, they affect substrates through electron transfer. The systematic name is according to donor/acceptor oxidoreductase [18]. Among high-potential enzymes used in organic chemistry, there are redoxes ones (oxidoreductases) which can ameliorate the oxygenation of C – H bonds. The asymmetric reduction of carbonyl functional groups to amines and alcohols can also be catalyzed by them. Pyridine nucleotide cofactor is required for most of biocatalysts in this group for catalysis. $NADP^+$ and NAD (P)H are so expensive thus using their stoichiometric amount is not economically possible and hence different *in situ* reproduction processes have been designed until make it possible to use catalytic quantities. Moreover, for decreasing the cost of stereo selective transformations by these enzymes, cofactor reproduction streamlines isolation of yields, barricades challenges of yields inhibition by cofactor, and can lead thermodynamically unfavorable transformations by conjugating to favorable reproduction processes [122]. In generally, to catalyze organic reactions, they need nonprotein groups (cofactors). Although redox biocatalysts have prosthetic groups to comfort processes, many biocatalysts explored for bioproduction required to interact with cofactors are not bridled to enzymes permanently. The most extensive lycaptived cofactors are various organic molecules that are usually referred to as coenzymes like ATP, NADP(H), and NAD(H). NADP(H) and NAD(H) have been particularly studied widely since 2000s for chemical processing applications [118].

Cofactor regeneration is carried out by developed methods involving chemical, photochemical, electrochemical, enzymatic, and microbial processes. Among them, enzymatic processes particularly are preferred for industries owing to their high efficiency and selectivity. There are two paths to obtain biocatalytic regeneration (Figure 33). The first one is via using substrate-coupled systems, in which an enzyme that uses both the oxidized and reduced type of cofactors is employed for catalyzing both benign product production from first substrate and the regeneration reaction of cofactor with a second one. Atypical example is the alcohol dehydrogenase (ADH)-catalyzed synthesis. NADPH-dependent ADH from *Thermoanaerobacter* sp. was used for enantioselective reduction of acetophenone to (*S*)-1-phenylethanol and the 98% conversion of acetophenone could be obtained when 2-propanol was applied as the secondary molecules to drive the catalyzed regeneration of NADPH by the similar ADH in batch reactors [118].

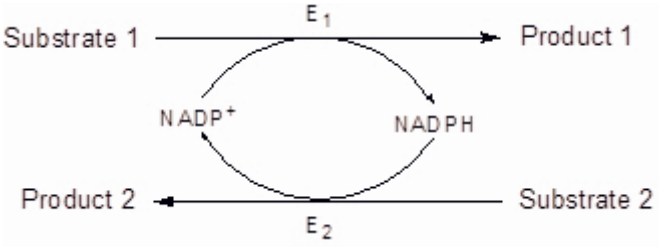


Figure 33. Enzymatic regeneration of cofactors [118].

Next way is achieved by applying a second enzyme to catalyze the cofactor regeneration reaction. The advantage of using the second enzyme is that, it usually provides us with more options of substrates for the cofactor regeneration reaction, therefore, by far facilitates the achievement of large thermodynamic driving forces for both reactions that is why it has been adapted for the majority of cofactor regeneration processes. Either way, producing value-added products from the second substrates applied for cofactor regeneration is desirable; otherwise, there is no option but to provide very cheap second substrates or regenerating them for reuse. For the latter case, usually electrochemical reactions are

applied, which means the second substrate applied for cofactor regeneration is regenerated electrochemically. In order to do so, the second substrates, namely, mediators, must possess both good electrochemical reactivity on electrodes and good reactivity with the enzyme and cofactor (Figure 34(a)). Cofactors can be directly regenerated on electrodes surface with no biocatalysts and mediators (Figure 34(b)). It may be told that this process uses as electrochemical cofactors regeneration. In mediator-assisted reproduction, an enzyme-catalyzed method can be applied to cofactor regeneration, while the mediator is reproduced on the surface of electrodes. These mediators have often more activity than cofactors for this method, and therefore the possibility for further reaction rates can be provided by them [118].

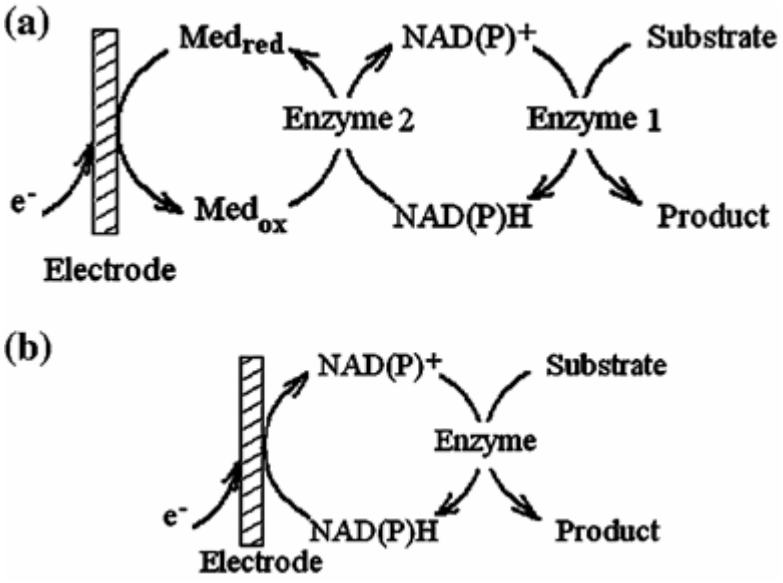


Figure 34. Cofactor regeneration with electrochemical reactions: (a) electro-enzymatic cofactor regeneration; (b) electrochemical cofactor regeneration [118].

The applicability of an *in situ* regeneration of cofactor is usually measured based on turnover numbers (the definition of TN is as follows: the number of generated product moles per cofactor mole per unit time) and total turnover numbers (the definition of TTN is as follows: the number of generated product moles per cofactor mole within the course of a process) of cofactor. 103 to 105 TTNs can be enough to economically make an organic process sustainable based on the amount of products [122]. An efficient regeneration of cofactors is benign in biosynthesis. It is so difficult to obtain a high total turnover number (TTN) of the cofactors, however, if the yields accumulate in reaction medium and provide high thermodynamic resistance verses forward processes. Accordingly, continuous flow reactors (Figure 35) that make frequency nutrition of reactants and removal of yields possible are usually preferable in large-amount synthetic usages. While the application of continuous flow reactors is vast and it is used with non-cofactor dependent biocatalysts, it has been a dismaying work with cofactor-associated processes as the cofactors usually left the reactors along with products and reactants [118].

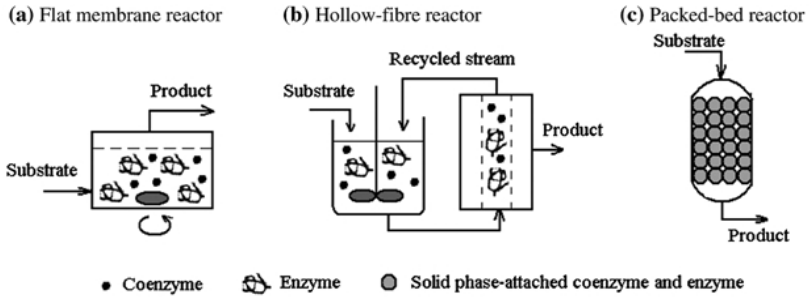
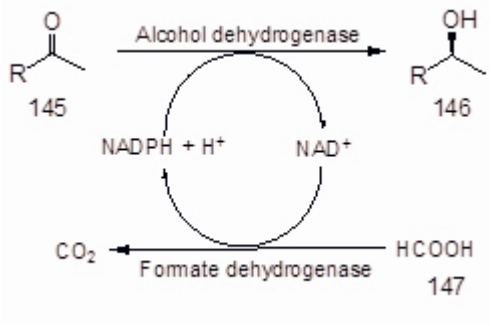


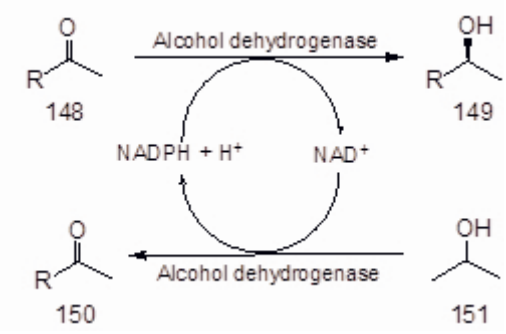
Figure 35. Typical configurations for sustainable cofactor retention in continuous-flow bioreactors [118].

Here, the hydrogen donor is the oxidizing substrate and the enzyme is called a dehydrogenase. When the molecular oxygen (O_2) is the acceptor, the enzymes will be called oxidase. All oxidoreductases depend

on cofactors since it either supply or take the reducing or oxidizing equivalent. The most required cofactors are $NADH/NAD^+$, $NADPH/NADP$, $FADH/FAD^+$, ATP/ADP, and PQQ. An effective cofactors regeneration approach is needed for affordable industrial processes, since most of them are expensive. Using second biocatalysts (the best method for NADH, is to apply formate dehydrogenase which employed formate and generates CO_2 (Figure 36(a)) or employing a second reactant is possible, if an isolated enzyme is being used (Figure 36(b)) [18].



(a)



(b)

Figure 36. Different approaches of cofactor regeneration [18].

A high number of alcohol dehydrogenases like an enzyme that obtained from *Lactobacillus kefir* (*LK*ADH), represent a wide substrate spectrum and good stereo selective features. Both enzymes and

correspond cofactors are usually unstable in organic medium or the interface of aqueous-organic biphasic solution. Moreover, the hydrophilic cofactors $NADPH + H^+$ or $NADH + H^+$ need aqueous medium for an easy propagation between catalytic sites. Therefore, for the conversions of low water soluble structures in organic media, contrary to a lot of alter biocatalysts, alcohol dehydrogenases cannot be used despite the fact that they frequently accept a relatively wide range of such substrates [123].

The catalyzed oxidation methods by enzymes have potential within a wide diversity of industrial applications such as food, textile, and pulp and paper industries. Molecular oxygen as electron acceptors is applied for enzymes recycling and this is the most interesting one. Therefore, laccaseeare particularly promising biocatalyst for aims that mentioned above [40].

There are non-heme diiron biocatalysts that have natural irregular effects in many secondary metabolites biosynthesizes. For instance, the synthesis of cephalosporin C antibiotic in eukaryotes applied a solitaire biocatalyst with an only one active site, to catalyze two variant oxidative processes an oxidative ring extension of the five-membered ring to a six-membered, and methyl hydroxylation (Figure 37). Single amino acid substitutions can inactivate either activity. In the contrast, synthesis of cephalosporin in prokaryotes employed separate biocatalyst for couple steps, but both of them are related to the bifunctional one in eukaryotes [124].

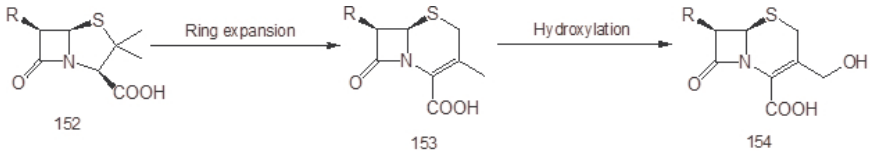


Figure 37. A non-heme iron (II) and 2-oxoglutarate-dependent cephalosporin C synthase in eukaryotes catalyzes two different catalytic steps with the same active site [124].

10. Conclusion

Enzymes are natural catalysts found in plants, animals, and micro-organisms where they catalyze processes that are pivotal to living creatures. Their application involves numerous biotechnological processes, such as the production of cheese, beer, and wine, that dates back to the starting point of human civilization. Biocatalysis presents many noble features in the field of Green Chemistry and synthesis of chiral building blocks causes to synthesis the enantiopure drugs or food additives: gentle reaction conditions with good regio-, chemo-, enantio-, diastereo-, and prochiral selective acylation/deacylation reactions. Application of enzymes in organic synthesis provides advantages in comparison with conventional chemical methods, such as mild reaction condition, high selectivity, high catalytic efficiency, and high product purity and quality [125, 126]. Asymmetric synthesis and kinetic resolution to obtain pure enantiomers are the most common applications of these biocatalysis and they have been used as chiral catalysts within the tool kit of the organic chemist. The transformation of macromolecules to energy and new materials, besides for growth, repair, and maintenance of cells are carried out by enzymes. Their capability to catalyze the products' formation in high enantiopurity is of the utmost significance in several industries by an exquisite stereochemical control. With contrast to the situation with natural enzymes, there is currently an almost complete lack of structural characterization or molecular modelling studies with dendrimers. In the future, these are needed to establish structure-function relationships for dendrimers on a firm basis and make more developments possible.

Acknowledgement

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