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Review

Enzyme Immobilization Technologies and Industrial Applications

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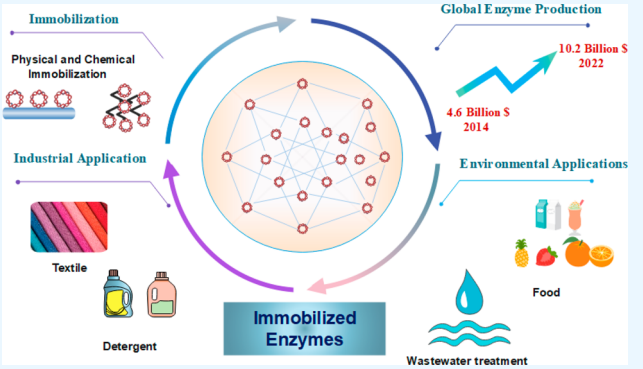
ABSTRACT: Enzymes play vital roles in diverse industrial sectors and are essential components of many industrial products. Immobilized enzymes possess higher resistance to environmental changes and can be recovered/recycled easily when compared to the free forms. The primary benefit of immobilization is protecting the enzymes from the harsh environmental conditions (e.g., elevated temperatures, extreme pH values, etc.). The immobilized enzymes can be utilized in various large-scale industries, e.g., medical, food, detergent, textile, and pharmaceutical industries, besides being used in water treatment plants. According to the required application, a suitable enzyme immobilization technique and suitable carrier materials are chosen. Enzyme immobilization techniques involve covalent binding, encapsulation, entrapment, adsorption, etc. This review mainly covers enzyme immobilization by various techniques and their usage in different industrial applications starting from 1992 until 2022. It also focuses on the multiscale operation of immobilized enzymes to maximize yields of certain products. Lastly, the severe consequence of the COVID-19 pandemic on global enzyme production is briefly discussed.



1. INTRODUCTION

Enzymes are protein catalysts that are omnipresent in animals, plants, and several microorganisms. Enzyme usage dates back to ancient times where these were primarily used in the manufacturing of many foods, e.g., yogurt, cheese, wine, beer, sourdough, and vinegar, besides being used in the manufacturing of commonly used products (such as linen, leather, textiles, etc.).[1] Additionally, enzymes have long been used to ease the intricate production steps/procedures of several industrial sectors. Nowadays, the demand for sustainable processes has extensively augmented the application of enzymes as catalysts in several industrial sectors.[2] In addition, the growing technological developments in protein extraction/purification together with novel developments in protein engineering have led to efficient manufacturing of certain enzymes with highly enhanced and tailored characteristics at an analytical purity grade.[3] Usage of enzymes in diverse industrial sectors is constantly growing, particularly throughout the previous two decades. These include dairy products' manufacturing,[4] starch transformation,[5] baking,[6] and beverage production (e.g., wine, beer, fruit concentrates, etc.).[3] Enzymes are also used in the textile industry in multiple manufacturing steps and purification procedures.[7] Additionally, the use of enzymes has become integral to the paper making,[8] cosmetics,[9] health care,[10] chemical manufacturing,[3] and detergents industries.[11] Moreover, the application of some enzymes in particular novel industrial sectors (e.g., biosensor manufacturing) is needed because of their high specificity to their target biomarkers. Also, enzymes have been frequently used in biofuel manufacturing and in the treatment and recycling of wastewater.[11]

Nevertheless, the desired useful properties of enzymes and their prevalent applications are hampered by their short shelf life, poor stability, and extreme sensitivity to many processes' settings/conditions.[12] Many of the disadvantages could be stopped or alleviated through immobilizing techniques.[13] The emergence of immobilizing enzymes has been an attractive topic since the 1960s.[14] The idea of enzymes' immobilization was introduced by Nelson and Griffin in 1916 after they noticed that invertase can hydrolyze sucrose after being absorbed onto charcoal.[15] Since then, several reversible/irreversible methods of enzyme immobilization were introduced that can enhance the physicochemical properties of enzymes, enabling them for applied usages.[16] The reversible immobilization of enzymes entails adsorption, metal binding, and ionic binding. Yet, the irreversible immobilization of enzymes comprises entrapment and covalent binding.[13]

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Besides the mode of immobilization, the carrier itself is key in the success of the methods. The carrier should possess good stability, entail considerable porous structures, and have a large surface area to maximize the immobilization efficiency. The carrier material should also be easily modified to facilitate enzyme immobilization. Additionally, it should be of a low cost, abundant, and environmentally friendly.[13] The development of enzyme immobilization strategies has led to the production of tailored enzymes that are robust and stable. They also enabled recovery and reuse of enzymes, reduced contaminants in products, and improved control of industrial processes.[11]

This review presents an updated overview of enzyme immobilization technologies, applications of enzymes in various industrial sectors, and the world's market of industrial enzymes. Enzyme immobilization methods, their advantages and disadvantages, and the impact of COVID-19 on worldwide enzyme fabrication are also discussed.

2. USEFUL ENZYMES FOR INDUSTRIAL APPLICATIONS

2.1. Lipase. Author: Lipases are widely present in most of the earth's fauna/flora and are abundant in fungi, yeasts, and bacteria.[17,18] Lipases are hydrolases that catalyze the conversion of triacylglycerols to fatty acids and glycerol. Lipase enzymes catalyze transesterification, interesterification, and esterification reactions. Lipase substrates are varied, including lipids, phospholipids, ether lipids, lysophospholipids, etc. This class of enzymes can undergo vital functions in transportation, digestion, and processing of dietary lipids.[17] Lipases, from microbial origins, are adaptable enzymes that perform a large range of bioconversion reactions, namely, aminolysis, interesterification, alcoholysis, esterification, hydrolysis, etc. Their distinctive characteristics include substrate stereospecificity, specificity, and regioselectivity.[18] Lipases present enantioselective catalytic properties and region selective capabilities. They are used in the production of emulsifiers, flavors, fragrances, cosmetics, thermoplastics, agrochemicals, and many other commonly used products. Lipases are also applied in the fabrication of several biodiesels by triglyceride transesterification, in addition to the manufacturing of concentrated fatty acids by means of fat hydrolysis.[18] Lipases can furthermore be used in the extraction of pitch from the pulp created during paper production steps, in the extraction of fats from milk products (e.g., cheeses and yogurt), in the extraction of impurities from cotton before it is further processed and dyed, and in the extraction of fats/fats' derivates during leather making processes.[17] Unfortunately, not all classes of lipases are able to operate at elevated temperature (i.e., 100 °C); besides that, the half-lives of the enzymes are conveyed to be very concise.[17] Consequently, the production of extra stable lipase forms that can endure the high production temperatures and have a longer shelf-life is desirable. Immobilization is one of the approaches that is adequate for attaining stable lipase enzymes.

2.2. Protease. Proteases are widely present in all animals, plants, and viruses, as well as in some microorganisms. Proteases represent an integral constituent of the biochemical processes that take place inside animals, humans, plants, and some microbes.[19] Proteases can be isolated and purified from living organisms, presenting high substrate specificity and efficient catalytic activity.[20] Proteases catalyze proteolysis, breaking down proteins into amino acids and/or small polypeptides. These classes of enzymes perform that mainly by means of cleavage of the peptide bonds in the proteins through hydrolysis reactions.[19] Proteases are involved in diverse fundamental biological functions, such as protein catabolism, digestion of ingested proteins, and cell signaling. Diverse types of proteases are able to catalyze similar reactions through entirely dissimilar mechanisms. Moreover, proteases have an important function of being involved in the turnover of cellular protein. Proteases can perform selective modifications of the proteins through limited cleavages like the activation of zymogenic enzymes, blood clotting, and processing of secretory proteins throughout the membranes.[20] Proteases are mostly categorized into endopeptidases that work by cleaving the peptide bonds inside proteins/exopeptidases that separate the amino acids from endings of proteins instead.[19] These enzymes comprise ∼60% of the global enzymes' market. A huge variety of proteases have drawn attention to exploit their biotechnological and physiological functions.[19] Proteases are crucial to several industries that require the enzyme-aided/digestion of proteins from different sources,[21] e.g., pharmaceutical, dairy, leather, food, baking, textile, and brewing industries.[5] Furthermore, proteases are used in various forms of medical therapies and are considered an important biocatalyst that is used in laboratories, medical care institutions, and hospitals. There is also significant attention on the examination of protease classes that are capable of catalyzing reactions in cold water, which will ease their application for detergent use in tap water, i.e., room temperature.[20] Proteases have very short half-lives, and thus immobilization is needed for attaining a stable form of the enzyme to widen and ease their worldwide application.

2.3. Amylase. Amylases are found in plants and animals; they catalyze hydrolyzing starch into sugars.[22] Amylases obtained from microorganisms possess an extensive spectrum of functions due to having more stability than those obtained from plant and animal sources.[23] Additionally, amylases from microorganisms' origins have a big production capacity and produce enzymes of certain required properties.[24] Amylases are glycoside hydrolases that work on α-1,4-glycosidic bonds.[23] They have 3-D structures that have the ability to bind to substrates and support the breakage of glycoside links.[23] Amylases comprise ∼30% of the consumption of enzymes globally.[22] The fabrication of amylases take place by means of solid-state fermentation or submerged fermentation. The manufacturing technique depends on certain physicochemical factors. For instance, submerged fermentation is applied for the manufacturing of amylases due to the simplicity of manipulation of parameters, e.g., temperature, pH, oxygen transfer, aeration, etc.[24] Currently, diverse microbial amylases are available commercially. These are applied in the food, detergent, fermentation, textile, and pharmaceutical sectors, etc.[23] Amylases are applied in starch manufacturing for the hydrolysis of starch to transform starch to a syrup of glucose, fructose, etc.[22] These are significantly applied in the food processing sector, e.g., for preparing digestive aids and starch syrups.[23] In the paper industry, amylases are used for the amendment of the starch in coated papers through the manufacturing of high molecular weight and low-viscosity starch.[24] Last but not least, amylases could be potentially useful for the manufacturing of fine chemicals. Nevertheless, the applications of lipases are often obstructed by their low stability, short shelf-storage life, and extreme sensitivity to

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process conditions.[22] These drawbacks can be greatly lessened by means of immobilization approaches.

2.4. Cellulase. Cellulases are produced mainly from bacteria and fungi, and these catalyze most cellulolysis reactions. Cellulase enzymes break down cellulose molecules to oligosaccharides and beta-glucose.[25] Cellulose breakdown is of important economic significance as it is a main component of plants that are being consumed. Because cellulose's molecules bind strongly together, cellulolysis is comparatively hard when compared to the breakdowns of other polysaccharides, e.g., starch.[26] Diverse classes of cellulases exist that differ both mechanistically and structurally.[26] Cellulase enzymes are crucial in several industrial sectors. They are used in undesirable color extractions from fruit juices and pulps. Cellulases are also used in the detergent industry as color brighteners and softeners, as well as in the biostoning of jeans products. Additionally, these enzymes are used in pretreating biomass for improving the nutritious value of food, as well as in treating industrial waste.[26] They also possess a broad-scale application in pharmaceutical, animal feed, textile, and paper processing industries, which makes them ranked as one of the most significant worldwide enzymes.[25] Nonetheless, using cellulase in all of these industries is restricted because of their reduced stability, concise shelf-storage life, extreme sensitivity to many of the processes' conditions, etc.[25] These drawbacks can be greatly reduced by means of immobilization approaches of cellulases.

3. PROBLEMS AND LIMITATIONS ASSOCIATED WITH INDUSTRIAL ENZYMES

Despite the vast advantages associated with using enzymes compared to traditional catalysts, some complications arise with their utilization in their raw forms.[27] The usage of enzymes, in some large-scale applications, is not the optimum choice for catalysis because these organisms are typically unstable, possess a short shelf-life, and may get inactivated easily by several mechanisms.[28] Enzymes are also highly sensitive to the various harsh process conditions (e.g., elevated temperature, extreme pH, etc.), besides the fact that several technical problems arise with the usage of enzymes in industrial applications, which makes them practically unreliable. For instance, most types of enzymes operate better when dissolved in water in homogeneous catalysis systems unlike the conventional heterogeneous chemical catalysts.[27] Additionally, traditional methods for enzyme recovery and reuse are very difficult to attain.[28] As shown in Figure 1, these diverse problems can be prevented or at least lessened through using different immobilization techniques that will be addressed in detail in the subsequent section.[28]

4. IMMOBILIZATION OF ENZYMES

Enzyme immobilization has been a captivating research topic since the 1960s.[14] Immobilization technologies have developed progressively but reached a plateau recently.[27] However, the recent expansion of biotechnological/nanotechnology advances has revitalized interest in the immobilization strategies of enzymes to a great extent.[29] Immobilization strategies entail fixing or entrapping enzymes within solid support materials. Researchers have suggested several support materials, besides many beneficial approaches for the immobilization of enzymes.[30] The main function of the support is to stabilize the structures of enzymes and accordingly preserve their

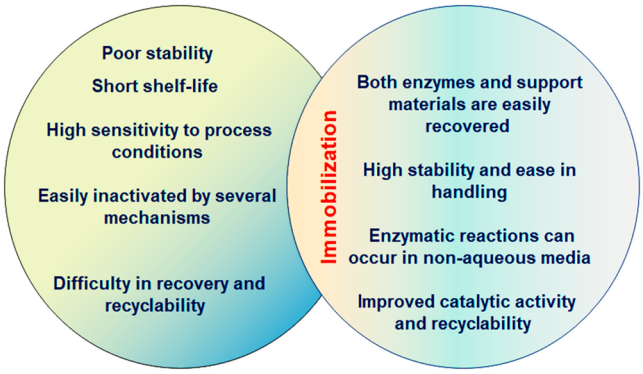

Figure 1. Problems associated with enzyme usage (left), and the advantages of immobilization processes (right).

efficacy to a great extent by rendering them more resistant to the surrounding environments.[31] Enzyme immobilization permits an easy recovery of both the used enzymes and their support materials, and this is particularly beneficial in the food, medical, and pharmaceutical applications.[13] Enzymes in their immobilized forms possess much higher stability and are also easier to handle when compared to their free forms.[32] Additionally, the enzymatic reaction can occur in a non-aqueous medium where the solid supports preserve the enzyme's constituents and make them stronger, which enhances their catalytic activity and renders them reusable for several times.[33] Another benefit of the immobilization process is that the catalysts can alter from homogeneous to heterogeneous forms after the enzymatic binding, which assists in separating the enzymes, producing products with high purity.[34] Various immobilization methods have been applied so far including encapsulation, cross-linking, covalent binding, adsorption, and entrapment, as displayed in Figure 2.[35]

4.1. Covalent Binding. Covalent binding is a well-established technique of enzyme immobilization that is

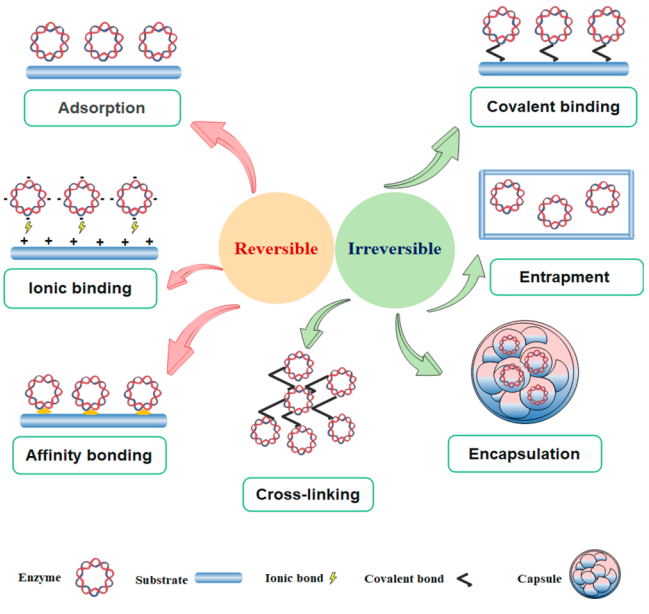

Figure 2. Diagram illustrating major enzyme immobilization methods. Reversible methods include adsorption, ionic bonding, and affinity bonding. Irreversible methods include covalent binding, entrapment, encapsulation, and cross-linking.

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accomplished by connecting the enzymes with support materials (e.g., porous silica, polyacrylamide, agarose, porous glass, etc.) via highly stable, strong linkages (Figure 2).[36] Covalent binding possesses many advantages, such as producing durable enzymes and obtaining a sufficient recovery of the enzymes as to be reused. Covalent binding increases the stereospecificity of the enzymes, elevating their stability.[37] For instance, covalently bonded lipases with glyoxyl agarose showed a 3-fold enhancement in their enantioselectivity.[37] By applying this method, there is a low probability of enzyme leakage even with the presence of high concentrations of substrates or along with strong buffer solutions, which usually facilitates many kinds of denaturation reactions.[35] The covalent binding basically takes place by activation of the support via adding reactive molecules then altering the polymer's backbone to activate the whole matrix.[38] The hydrophilic polysaccharide polymers are the most commonly used supports in this strategy.[13] In addition, electrophilic groups are also used and are produced on the support material in the beginning of the reaction, which develops strong interactions with the nucleophiles of the proteins.[39] Several functional groups of amino acids are also suitable for usage in this approach, such as the carboxylic group of aspartic acid, hydroxyl group of threonine, amino group of arginine, and sulfhydryl group of thiol, besides imidazole and indole groups.[39] In addition, carriers having an epoxy group are used as they react easily with the amino groups under mild conditions, producing stable bonds.[39] Unfortunately, some limitations arise with applying the covalent binding, for instance, this strategy requires relatively intricate steps and long incubation durations for proper immobilization to occur.[40] Sometimes, extra modification in the enzyme's structure is needed to expose some suitable functionalities for the covalent binding to occur, and these alterations in the reaction media may lead to denaturation of the enzymes. Additionally, typically a low quantity of immobilized enzymes is bound to carriers (~0.02 g/g carrier), which is not favorable for wide-scale industrial applications.[40] Furthermore, some restrictions in the mobility of the enzymes that are attached to the carriers might take place, which can restrict some conformational changes needed during catalysis, resulting in lowering the enzyme activity.[41] Nevertheless, the advantages of this immobilization strategy outweigh its drawbacks, especially because it obtains an augmented enzyme stability and a sufficient recovery/reusage of the enzymes.[13]

4.2. Cross-linking. Cross-linking immobilization is a strategy where enzymes are interconnected through covalent bonding without carriers.[42] The intermolecular cross-linking is accomplished through the presence of linker agents, which are used as bridges between two adjacent enzyme molecules, as presented in Figure 2.[42] Cross-linking immobilization delivers a robust connection between the enzymes, leading to superior stabilities. Several types of cross-linking strategy were reported, e.g., cross-linked spray-dried enzymes, cross-linked aggregates, cross-linked dissolved enzymes, etc.[36] Cross-linked dissolved enzymes refer to intermolecular cross-linkages of enzymes in their crystal forms using glutaraldehyde. It is a distinguished active immobilized enzyme technique that produces controllable particle sizes (1−100 μm). It possesses significant resistance to organic solvents and elevated temperatures. Its high stability, recycling with optimum catalytic activity, and volumetric efficiency make it highly desirable for industrial biotransformations. However, this strategy is very expensive because it requires the enzymes to be in a crystallized form, which necessitates obtaining a highly pure enzyme.[43] Aggregates of cross-linked enzymes are formed through straightforward enzyme precipitation in an aqueous media, which produces physical aggregates composed of the protein particles. This system is useful to many classes of enzymes because it permits a concurrent formulation of aggregates of the cross-linked enzymes, containing more than two enzymes.[43] The formed aggregates are attached by noncovalent bonds by maintaining the tertiary structures. Subsequently, cross-linkages of the aggregates make the enzymes perpetually insoluble, preserving their organized structures and consequently maintaining their catalytic efficiency. Forming aggregates of cross-linked enzymes is a significant strategy that is cost-efficient; however, it possesses poor mechanical stability.[43] Lastly, the cross-linked spray-dried enzyme method is also used in some industrial applications. However, its usage is restricted because spray-drying must be applied, which sometimes reversibly deactivates the enzymes.[44]

4.3. Adsorption. Immobilization by adsorption is a simple carrier bound technique where reversible immobilization is attained. This technique mainly depends on physical adsorption. Materials applied for the adsorption to occur include ion-exchange resins, alumina, activated carbon, and many more.[33] This method is relatively cheap and easily implemented, yet it possesses a weak binding force (i.e., hydrogen bonds, salt linkage, ionic bonds, hydrophobic bonds, etc.) among the carrier and the enzyme.[27] Depending on the arrangements of proteins and the charges of matrices, a strongly adsorbed/undistorted enzyme can be produced. In this strategy, any kind of carrier materials could be used, yet not all types of enzymes could be immobilized on any carrier material because, for an appropriate adsorption to take place, some conditions must be met, i.e., the affinity of the enzyme−carrier is of vital importance.[36] A successful adsorption is guaranteed with the presence of specific active groups that are present on the carrier material. These help in the development of interactions among the enzymes and carriers. Yet, if not present, these interactions could be modified via adding carrier modifiers that enable the connections between the enzymes and carriers, as displayed in Figure 3.[33] The advantages of enzyme immobilization by adsorption are that minimal activation steps are required, few reagents are needed, and it is a cheap method and easily implemented.[32]

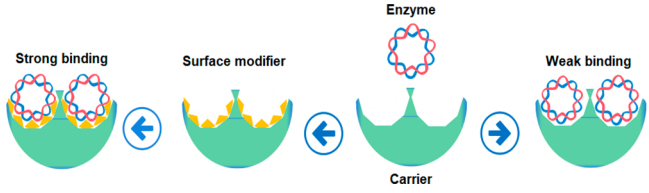

Figure 3. Effect of the surface modifier in adsorption immobilization. The surface modifier strengthens the binding of an enzyme to its carrier.

4.4. Ionic Bonding. Ionic bonding is a straightforward, inexpensive, and reversible immobilization technique that entails ionic interaction among the enzymes and the support materials. The nature of this noncovalent immobilization is that the procedure is simply reversed by altering the temperature as well as the ionic strength.[32] The support materials used in this immobilization technique are generally

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Table 1. Immobilized Enzymes for Food Applications

enzyme	immobilization method	support	application	results	ref
protease	entrapment	mesoporous silica and zeolite	milk coagulant for dairy products	immobilized protease converted milk into cheeses in <90 min	56
protease	cross-linking	chitosan beads	production of gluten-eliminated beer	gluten content was decreased from 65 to 15 mg/kg	57
amylase	covalent binding	chitosan	barley malt hydrolysis	increasing the product yield	58
pectinase	encapsulation	polyvinyl alcohol gel	apple juice clarification	80% turbidity reduction	59
pectinase	adsorption/cross-linking	polymeric matrix	apple juice clarification	immobilized pectinase was recycled 10 times	60
lipase	encapsulation	α-lactalbumin nanotubes	flavor enhancement for low-fat cheeses	immobilization enhanced the flavor of cheese	62
lipase	adsorption	hydroxyapatite	production of methyl acetate aroma	immobilized lipase activity was preserved after 3 h at 60 °C	63
β-galactosidase	covalent binding	chitosan/silica	lactose hydrolysis in dairy products	high efficiency of lactose hydrolysis reaching up to 62%	65
β-galactosidase	covalent binding	polyvinyl alcohol	lactose hydrolysis in dairy products	high efficiency of lactose hydrolysis reaching up to 75%	66
β-galactosidase	encapsulation	calcium alginate	hydrolysis of cheese whey lactose	high efficiency of lactose hydrolysis reaching up to 72%	67

charged, as the proteins should have opposite charges in order for them to bind together.[45] The ionic bonding is simply reversed by changing the pH or through salting out of the enzymes. To sustain an optimum pH throughout the reaction, easy control of the acidity/alkalinity in the mixture is undertaken, as the matrix that immobilizes the enzyme is steadily charged. The occurrence of a charged support produces some complications, such as distortion of the enzyme structure, changes in the enzyme kinetics, etc. Besides that, excessive charges can deteriorate the enzyme catalysis, which may thus hinder obtaining a high product yield.[32]

4.5. Entrapment. Entrapment is an irreversible immobilization process. It is simply described as caging of enzymes in a network of fibers, through covalent or noncovalent bonds.[46] It is also described as the entrapment of enzymes inside a support material or fiber, either having a lattice structure or in the membranes of polymers.[47] In using this method, the enzyme leakage can be easily avoided through controlling the pore size of the polymeric network that permits the free diffusion of the reaction contents, either substrates or products.[22] In this strategy, enzymes do not react with polymers; consequently, denaturation is generally prevented. The entrapment technique has diverse advantages including high loading capacity of the enzymes, low fabrication costs, enhanced mechanical stability of the entrapped enzymes, and lower mass transfer.[48] It also allows the modification of the encapsulation material to get an optimum microenvironment by attaining an optimal pH, suitable polarity, or amphilicity.[35] Gelation of polycationic or polyanionic polymers after adding multivalent counterions is the most used technique for the entrapping of enzymes.[49] A number of disadvantages arise when using this immobilization technique. For instance, a mass transfer opposition occurs as extension of polymerization increases the matrix thickness; accordingly, the substrate cannot diffuse deeply to reach the enzyme's functional sites.[50] Moreover, the entrapped enzymes could possibly leak out if the pore sizes of the support are large. Entrapment also possesses a low loading capacity of the enzymes, and the support might be ruined during the polymerization steps. Many procedures are applied for enzyme entrapment such as photopolymerization, sol−gel method, and electropolymerization.[50]

4.6. Encapsulation. The encapsulation immobilization technique involves entrapping several biomolecules into different polymeric matrices.[45] Encapsulation is analogous to entrapment in the way that both the enzymes and the cells are free in solutions yet in a controlled space. Encapsulation is aimed at immobilizing sensitive enzymes and cells' solutions bounded in tiny vesicles having porous membranes. Sizable enzymes cannot move out or into the capsules, yet tiny substrates/products could move freely across a semipermeable membrane.[51] Encapsulation maintains biological systems in a fine film so as to avert the biocatalysts from contact with the environment, which might damage their efficiency. Therefore, encapsulation allows the activity of biocatalysts to last for long periods.[52] Several support materials (e.g., cellulose nitrate and nylon) are used to manufacture microcapsules with sizes from 10 to 100 μm.[53] Ionotropic gelation of alginates and silica-based nanoporous sol−gel glasses have shown its efficiency as well for enzyme encapsulation. This method is distinguished by the fact that the enzyme can be encapsulated very easily. The development in material sciences added to the formed encapsulation with enhanced properties, e.g., augmented morphological stability, designed physicochemical permeability, and decreased enzyme leakage.[53] In addition, there is a possibility of co-immobilization, where enzymes could be immobilized in any needed combination to suit specific uses. However, some limitations are associated with this technique. For instance, the diffusion problem is severe and may cause rupturing of the membranes if the products from the reaction gather quickly.[53]

5. INDUSTRIAL APPLICATIONS OF ENZYMES

Nowadays, many industrial applications are dependent on enzymes in many of the industrial steps because enzymes have the ability to catalyze many types of chemical reactions.[49] An enzyme with a desired specified activity could be easily tailored by means of protein engineering so as to be used for a particular industrial purpose. Enzymes are applied in detergent, textile, pharmaceutical, medical, and food industries, etc. These are also widely applied for water treatment and in sewage/wastewater recycling as well.[23] Even though enzymes are very beneficial in most of the industrial sectors, by means of

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Table 2. Application of Immobilized Enzymes in the Detergent Industry

enzyme	immobilization method	support materials	stain type	results	ref
protease	covalent binding	Eudragit	blood and egg yolk	immobilized protease retained 76% of the initial tensile strength	70
protease	physical and covalent binding	mesoporous silica nanospheres	not reported	immobilized protease retained 63.6% activity after 1 h and the catalytic efficiency remained for 12 cycles	71
lipase	adsorption and cross-linking	glutaraldehyde	oil stain	80% of the lipase activity remained with using buffer solution	75
lipase	covalent binding	arylamine glass beads	oil stain	all of the used detergents performed better with the immobilized lipase	74
α-amylase	cross-linking	glutaraldehyde	starch	immobilized enzyme showed a starch hydrolysis efficiency of 15.5%	76

immobilization their benefits and range of applications can be maximized.[54]

5.1. Food Industry. Immobilized enzymes are broadly applied in the food sector due to various benefits including their high thermostability and endurance in the elongated food processing steps.[55] Several immobilization methods and different support materials were used for enzymes that are being used in the food sector, as illustrated in Table 1. Kumari et al.[56] immobilized alkaline protease onto mesoporous zeolite/silica to convert milk into cheese in a short duration (90 min). The catalytic activity was preserved to 74% following 16 storage days when compared to free proteases that preserved 50% of the initial activity.[56] Protease was also immobilized by cross-linking with chitosan and was used for eliminating the beer's gluten. Immobilization rendered the enzyme to be highly effective in reducing the original beer's gluten content of 65 mg/kg, reaching 15 mg/kg following 10 h of treatment.[57] Additionally, covalently immobilized amylase with chitosan increased the conjugate's thermal stability by 35%, improved the amylase's resistance to pH inactivation, and elevated the product yield of the hydrolysis of barley by 1.5-fold.[58] Many immobilized enzymes are being used in the processed juice production steps, for instance, pectinase immobilized using poly(vinyl alcohol) was used for clarifying apple juice where the turbidity reduction reached 80% after 3 cycles. The enzyme was reused 8 times, keeping 20% of its initial efficiency.[59] Then the same enzyme was used for the same purpose, but it was immobilized on recyclable polymers. The apple juice was effectively clarified, and the immobilized pectinase was reused 10 times with a negligible loss in activity, reported to be <5%.[60]

Immobilized lipase enzymes are widely applied in dairy manufacturing to enrich cheese flavors during fat hydrolysis and to accelerate the cheese ripening, besides being applied in butterfat lipolysis.[61] α-Lactalbumin nanotubes were used as carriers for lipases where they could release larger quantities (i.e., 1.5-fold) of free fatty acids compared to the free enzymes. That is, lipase nanotubes in low-fat cheeses revealed twice the quantity of free fatty acid release when compared to normal low-fat cheeses constituting similar contents of milk fat, thus creating a flavor-enhanced low-fat cheese.[62] Lipases are also used as aroma builders in the beverage industry because they are used in the synthesis of short-chain esters (methyl acetate) that are used as aromatic additives.[61] Substantial stabilization of lipases was attained after immobilization by adsorption onto hydroxyapatite to be used as methyl acetate food aroma. The immobilized enzyme's activity, in one of the research works, was preserved following 3 h at 60 °C, whereas free lipase lost 50% of the original activity after only 30 min.[63] β-Galactosidase is widely applied for hydrolyzing lactose in dairy products.[64] Immobilized β-galactosidase with chitosan/silica supports showed a high efficiency for lactose hydrolysis, reaching up to 62%. Besides that, with constant application in fixed-bed reactors (200 h), the system maintained 90% of its activity.[65] The same enzyme was immobilized using poly(vinyl alcohol) and revealed to efficiently hydrolyze lactose (75%) following 5−6 h. Besides that, the conversion degree was reduced to 50% following 30 uses.[66] Furthermore, calcium alginate was used for β-galactosidase immobilization, and the nanocomposites significantly increased the rate of lactose hydrolysis in cheese whey, reaching 72%.[67] All of these relevant studies have proven the significant role of the immobilized enzymes in the diverse food/beverage production sectors.

5.2. Detergent Industry. Enzymes have long been added to detergents' formulations for the removal of certain types of stains that ordinary chemicals cannot remove. Additionally, detergents that are enzyme-based can be applied in small quantities as they have the ability to remove stains at ambient temperatures. Proteases, for instance, are vital in the removal of blood, fish, egg, meat, and grass strains; besides that, these can efficiently remove the protein classes that are found in human sweat. Amylases, on the other hand, are added to detergent formulations to remove starch stains, e.g., gravies, cereals, potatoes, and chocolates. Lipases can effectively remove oil and fat stains. In addition to stain removal, enzyme-based detergents have other functions, e.g., cellulases augment the softening and improve the color brightening of cotton-based fabrics.[68] The immobilization of detergent enzymes improves the cleaning efficiency, preserves the enzymes' catalytic activity, and produces no harm on wool-based cloth, besides other advantages as displayed in Table 2. For instance, proteases hydrolyze the natural protein fibers (e.g., silk and wool keratins), which leads to irreversible damages to the garments' quality.[69] In this context, Vasconcelos et al.[70] investigated the efficacy of covalently bonded immobilized protease with Eudragit on wool. Immobilized protease kept 76% of the original tensile strength, whereas the samples with free enzyme retained 37%. In addition, the application of the immobilized enzyme displayed no damages on the fibers of wool.[70] Besides being lenient on wool, an immobilized protease fabricated nanoenzyme preserved a substantial catalytic efficacy for 12 cycles and also retained 63.6% activity after 1 h at 60 °C, while free enzymes lost their whole activity.[71]

Lipases are the second most important detergent enzymes after proteases.[72] Lipases are added to laundry and dishwashing detergents specifically for oil stain removal, and they operate under diverse temperature/pH ranges.[73] Nevertheless, lipases possess low cleaning effectiveness in normal water, due to the mass transfer barriers among the enzymes and oils.[74] In this context, lipases were immobilized by adsorption/cross-linkages using glutaraldehyde, where the experiment on woolen cloth exhibited excellent oil stain removal ability, and 80% of the

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catalytic efficiency was preserved following the washing cycle.[75] Lipases were also immobilized using arylamine glass beads, and the conjugate effectively removed oil stains from cotton clothes. Retention of the immobilized enzyme initial activity reached up to 75.59% and had the ability to be reused up to 100 cycles.[74] Shukla and Singh[76] immobilized α-amylase on six dissimilar matrices by applying ionic binding, adsorption, and entrapment. Glutaraldehyde cross-linker was the best matrix applied, showing the highest operational stability. The hydrolysis effectiveness of the immobilized α-amylase was reported at 15.55%, proving that it is proficient for starch stain removal from cloth.[76]

5.3. Textile Industry. A considerable increase in the usage of enzymes in the textile sector has taken place recently.[69] Enzyme immobilization techniques are highly preferable though, even in the textile sector, because they stabilize the enzymes to a great extent and prolong their shelf life, as presented in Table 3.[77] For instance, keratinase was immobilized using chitosan-β-cyclodextrin through cross-linking, and the final conjugate showed high thermal stability (70 °C). The storage stability was significantly enhanced, reaching up to 53.5% following 30 days when compared to the free ones.[78] Furthermore, cellulases are widely used in the textile processing steps due to their capability to modify cellulosic fibers, generating higher-quality textiles.[79] However, cellulase enzymes have a poor recovery/reuse; subsequently, it was proposed to immobilize cellulases onto kaolin by covalent bonding/adsorption to advance the knitted fabric quality. Immobilization has provided an excellent pilling resistance to the fabric with better tensile strength, and it gave a better recovery/reuse of the cellulases for 3 successive cycles.[80] Yu et al.[81,82] immobilized cellulase enzymes with Eudragit L-100 and Eudragit S-100 by noncovalent immobilization. The immobilized enzyme resulted in higher stability and preserved 51% and 42% of enzyme's efficiency by the third cycle for Eudragit S-100 and Eudragit L-100, respectively. Results of bending stiffness revealed that the fabrics treated with immobilized enzymes were softer than the control samples. Sankarraj and Nallathambi[83] immobilized cellulase enzymes by adsorption on calcium alginate starch where less weight loss was observed by the researchers. Besides that, minimum lessening of the tensile strength and a greater index of whiteness were achieved.[83] Also, immobilized cellulase with epoxy resin was used in biopolishing of fabrics for 6 cycles effectively with no tensile strength loss.[84] In addition, chitosan was coated using poly(vinyl alcohol), and cellulases were immobilized on it; 52% of the enzyme's activity was retained after 8 cycles.[85]

The wool processing industries apply environmentally harmful chlorination procedures to achieve shrink-resistant wool end products.[69] The addition of proteases instead of the chlorination process showed promising shrink resistance outcomes, while concurrently augmenting the wool whiteness, ease of handling, and dyeability; most importantly, it did not harm the ecosystem.[86] In spite of these advantages, the protease enzyme treatment may lead to certain damages to the wool fibers.[87] Immobilization of proteases, on the other hand, elevates the molecular sizes of enzymes, limiting the proteolytic attack to cuticles and causing no damage to the wool. In this context, proteases' pattern of diffusion into wool, covalently binded to polyethylene glycol, was studied. It was observed that the amended protease stayed at the surfaces of the cuticle layers, consequently creating an elevated tensile strength and less fiber felting.[88] Also, Silva et al.[89] showed that Esperase protease, covalently bound to Eudragit S-100, retained 45% of

Table 3. Immobilization of Enzymes for Use in Textiles

enzyme	immobilization method	carrier	storage stability and reusability	ref
keratinase	cross-linking	chitosan-β-cyclodextrin	storage stability of 53.5% after 30 days	78
cellulase	adsorption and covalent bonding	kaolin	better recovery/reuse for 3 cycles	80
cellulase	noncovalent binding	Eudragit S-100 and Eudragit L-100	stability of 51% and 42% after 3 cycles	81, 82
cellulase	adsorption	calcium alginate starch bead	ND[a]	83
cellulase	adsorption	epoxy resin	reuse for 6 cycles	84
cellulase	adsorption	polyvinyl alcohol coated chitosan	stability of 52% after 8 cycles	85
protease, Esperase	covalent/cross-linking	Eudragit S-100	activity of 72% after 5 cycles	89
laccase	covalent binding	green coconut fiber	stability 45−50% after 4 cycles	91

[a]ND = not detected.

Table 4. Immobilized Enzymes for Dye Removal from Water

enzyme	immobilization approach	support	removal efficiency	storage stability and reusability	ref
laccase	cross-linking	Cu(II) ion chelated chitosan	43%, 69%, and 87% degradation of methyl orange, cibacron blue, and reactive black 5, respectively	81% after 20 uses	95
laccase	covalent binding	glycidyl methacrylate functionalized polyacrylamide alginate	dye removal of 55%	50% after 5 cycles	96
laccase	entrapment	alginate bead	dye removal of 66%	95% after 15 days	97
laccase	adsorption	TiO_2−ZrO_2−SiO_2	100% alizarin red S removal	90% after 20 days	98
manganese peroxidase	covalent binding	Fe_3O_4/chitosan	98% and 96% of reactive orange 16/methylene blue	86% after 5 cycles and 60% after 14 days	99
manganese peroxidase	cross-linking	chitosan beads	97% dye removal	60% after 10 cycles	100
horseradish peroxidase	adsorption/cross-linking	polyaniline grafted polyacrylonitrile	91% for direct blue 53 and 95% for direct black 38	83% after 8 weeks	101
horseradish peroxidase	cross-linking	kaolin	76% removal of anthraquinone dye acid violet 109	15% after 4 cycles	102
horseradish peroxidase	adsorption and covalent bonding	polyamide 6	70% for reactive black 5 and malachite green	70% after 20 cycles	103

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Table 5. Immobilized Enzymes for Phenol Removal from Water

enzyme	immobilized approach	support	removal efficiency	storage stability and reusability	ref
tyrosinase	cross-linking	iron oxide nanoparticles	>70%	100% after 3 cycles and 58% after 7 cycles	104
tyrosinase	cross-linking	polyacrylonitrile beads	96%	78% after 6 cycles	105
tyrosinase	adsorption	sodium aluminosilicate	15−60%	ND[a]	106
laccase	covalent binding	zeolite	71−98%	50% after ∼3 weeks	107
laccase	adsorption	sodium zeolite Y	86.7%	60% after 14 days	108
laccase	covalent binding	Cu(II)-chitosan	>96%	50% after 8 cycles	109
laccase	covalent binding	epoxy functionalized silica	95%	61% after 5 cycles	110
laccase	cross-linking	magnetic nanoparticles	86−100%	83.5% after 6 cycles and 80% after 30 days	111
horseradish peroxidase	covalent binding	biocarbon	90%	>79% after 4 cycles	111
horseradish peroxidase	encapsulation	tyramine−alginate	96%	60% after 4 cycles	112
horseradish peroxidase	covalent binding	graphene oxide	92%	100% after 60 days	113
horseradish peroxidase	covalent binding	graphene oxide	100%	70% after 10 cycles and 97% after 35 days	114

[a]ND, not detected.

its stability at 60 °C. The efficiency of the conjugate was reported at 72% of the initial value following reuse for 5 times.[89] The immobilized system is thus a good alternative for wool shrink-resistance and could substitute the standard chlorine pretreatments. Laccase is being used excessively in the textile sector for the decolorization of textile effluents and in polymeric dye synthesis.[90] Covalent binding was applied for the production of immobilized laccase where fibers of green coconut silanized with 3-glycidoxypropyltrimethoxysilane were used as support. Immobilized laccase was used in textile dye decolorization, and the covalent binding enhanced the enzyme's thermal stability at a temperature of 50 °C. High efficiency in the decolorization of reactive textile dyes was achieved.[91]

5.4. Wastewater Treatment. Water pollution is an alarming obstacle that the entire world is facing nowadays. Water pollution causes several alarming problems, including threatening the public health, ruining diverse agricultural products, and having a negative impact on the world's economy. Discarding large quantities of untreated wastes from industries to water bodies is one of the principal reasons for water pollution.[92] Disposal of phenolics and dyes has raised extensive concerns lately from the scientific community due to their carcinogenic and mutagenic properties, toxicity, and poor biodegradability.[93] Many developments on dye and phenolics extraction through enzyme immobilization systems have been introduced recently and proven successful.

5.4.1. Dye Removal. Several factories produce dye-containing effluents that negatively affect seas, oceans, rivers, lakes, etc. Several enzymes, e.g., laccase, preoxidases, and azo reductases,[94] were reported to remove the dyes during the water treatment steps. However, these enzymes possess some limitations, especially losing their activity when used in their native forms. Hence, the immobilization techniques for enzymes are applied to overcome these drawbacks, as shown in Table 4. A cross-linked laccase chitosan bound to a metal ligand Cu(II) ion has been applied to degrade three types of dyes in wastewater where the removal efficiencies were reported at 69%, 87%, and 43% for cibacron blue, methyl orange, and reactive black 5, respectively. Besides that, the remaining efficiency of immobilized enzyme was reported at 81% after 20 water treatment cycles.[95] Another report discussed the effect of immobilized laccase on water effluents treatment using a mediator (i.e., glycidyl methacrylate functionalized polyacrylamide−alginate). Immobilization was done using covalent binding, and the removal efficiency was 55%.[96] Additionally, complex polymers of dyes were oxidized through immobilized laccase encapsulated in Cu-alginate beads displaying 66% reduction in the color of effluent water. The immobilized enzymes kept their activity at 95% for 15 successive days of storage.[97] Laccase adsorbed on TiO_2−ZrO_2−SiO_2 was used in the degradation of dyes from textiles. Immobilization efficiency was estimated at 96% for TiO_2−ZrO_2−SiO_2−laccase, while the degradation of dyes reached up to 77%, 100%, and 91%, for reactive black, alizarin red S, and remazol brilliant blue R, respectively.[98]

Manganese peroxidase has high efficacy in organic pollutant degradation. Various research studies have investigated the effectiveness of immobilized manganese peroxidase in wastewater treatment, as shown in Table 4. For instance, covalently binded manganese peroxidase with Fe_3O_4/chitosan showed significant efficiency in elimination of methylene blue and reactive orange 16, while its activity remained stable after 5 cycles of dye removal.[99] Another report investigated the efficiency of the same enzyme in dye removal, but this time it was cross-linked on glutaraldehyde/chitosan. The maximum dye degradation was achieved at 97%, and the conjugate retained 60% effectiveness after 10 repeated decolorization cycles.[100] Also, horseradish peroxidase enzyme was immobilized using polyaniline chains through attachment on films of polyacrylonitrile and then by cross-linking using glutaraldehyde. Immobilized peroxidase had an extraction effectiveness reported at 91% for direct blue 53 and 95% for direct black 38.[101] Šekuljica et al.[102] immobilized horseradish peroxidase on kaolin/glutaraldehyde, and they used the conjugate for decolorizing anthraquinone acid violet where 76% of decolorization was accomplished.[102] Another report investigated the effect of the same enzyme immobilized using polyamide 6 by covalent bonding and adsorption for dye decolorization. The highest dyes' decolorization (malachite green and reactive black 5) reached up to ≥70% efficiency.[103] Apparently, immobilization of enzymes can support the application of biocatalysts for continuous degradation of numerous environmental pollutants and dye removal from wastewater.

5.4.2. Phenol Degradation. Phenolic compounds are water pollutants that display high toxicity and low biodegradability. Application of durable and environmentally friendly enzymes, especially in their immobilized forms, is a good strategy for extracting phenolics from wastewater.[104] Several oxidoreductases, including laccase and tyrosinase, were immobilized for the removal of phenolics (Table 5) due to their high catalytic

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activity, high selectivity, good thermal stability, etc. For example, magnetic iron oxide nanoparticles coated with tyrosinase were used for phenolic compound extraction from wastewater where the nanoconjugate revealed a high ability of phenol removal, reported at 70%.[104] The same enzyme was cross-linked with polyacrylonitrile beads using glutaraldehyde, and the final conjugate extracted 96% of the total phenols (470 mg/L), whereas the free tyrosinase removed only 80%. Besides that, the immobilized system was reused for 6 cycles, and its activity was maintained at ∼78%.[105] Another report discussed phenol degradation using tyrosinase immobilized on siliceous support where the removal efficiency reached up to 60%.[106] Laccase has also been applied for phenol degradation but after certain modifications of the support materials. For instance, Ameri et al.[107] have chemically changed the zeolite support (i.e., to have a better pore size) to be covalently bound to laccase showing high effectiveness and reusage for 10 times for phenols degradation. The outcomes agree with those reported by Taghizadeh et al.,[108] who immobilized laccase using sodium zeolite Y besides its modified desilicated forms. The formed system displayed improvement in the immobilization efficiency reported at 94.50% and 74.39% for laccase-desilicated and laccase−sodium zeolite Y, respectively. Also, laccase showed high biodegradation ability of bisphenol A (86.7%) after immobilization.[108] Laccase was also immobilized on Cu(II) chitosan nanoparticles through adsorption, where >96% of the total phenols were extracted and the efficiency of the immobilized laccase stayed >50% after 8 cycles.[109] Moreover, epoxy-functionalized silica was used in laccase immobilization by covalent bonding. The system achieved significant removal efficiency (∼95%) for catechol and was reused for 5 cycles.[110] Another report used magnetic nanoparticles amended by dialdehyde starch as cross-linker in laccase immobilization which efficiently removed phenolics in wider pH/temperature ranges. The extraction was reported at 86.1%, 93.6%, and 100% for phenol, 4-chlorophenol, and 2,4-dichlorophenol, respectively. Efficiency retention of 83.5% was reported after 6 cycles.[111]

Horseradish peroxidase, especially in its immobilized form, is an important biocatalyst applied for phenol degradation in the treatment of wastewater (Table 5). In that context, peroxidase immobilized on the surface of biocarbon showed a phenol removal efficiency of 90%. After 4 cycles, the immobilized horseradish peroxidase preserved >79% activity with a phenol removal efficiency reported at 64%.[111] The same enzyme, but encapsulated into tyramine−alginate beads, revealed a removal efficiency of 96% and a substantial reusability reported at 60% after 4 cycles.[112] Likewise, graphene oxide was applied as a support for immobilization of horseradish peroxidase via cross-linking, and 92% of total phenols were degraded after 1 h of treatment.[113] This is consistent with the results reported by Besharati Vineh et al.,[114] who applied the exact conjugate displaying 100% efficiency for phenol degradation, and the reusability after 10 cycles retained 70% of the original activity. Therefore, immobilized enzymes can efficiently extract phenolic compounds in wastewater in short duration.

6. WORLD MARKET FOR INDUSTRIAL ENZYMES

The key drivers of the enzyme market include the innovative technologies introduced to improve the biocatalyst cost-effectiveness and efficiency. Additionally, there is a rising awareness from consumers of replacing synthetic chemicals and petrochemical products with natural and biodegradable ones.[115] Additional factors boosting the enzyme market progression involve the high demand from detergent producers, pharmaceutical factories, textile industries, animal feed producers, bioethanol producers, and cosmetic manufacturers for these environmentally friendly substitutes.[115] The global enzyme market was reported at $11.47 billion during 2021 and is estimated to increase at a compound annual growth rate of 6.5% starting from 2022 up to 2030. The global market of enzymes was evaluated to be $1.6 billion during 2002. These were divided among food enzymes reported at 29%, feed enzymes of ∼15%, and general technical enzymes at 56%.[116] Proteases are ranked first among all enzymes globally used,[115] whereas amylases represent almost 30% of the world's market.[117] Lipases represent ∼10% of the worldwide enzyme production as they catalyze several types of chemical reactions in either aqueous or nonaqueous medium.[118] In 2017, the lipase world market was developed at a yearly growth rate of 6.2% and expected to increase up to $797.7 million by 2025.[119]

The global amino acids market was reported at $26.1 billion during 2021 and is expected to increase at a rate of 7.4% starting from 2022 up to 2030. Also, the market is expected to have increasing demand for amino acids, especially for the nutraceutical/pharmaceutical sector, which necessitates the use of enzymes. The dietary supplements market was reported at $151.9 billion during 2021; besides that, it is anticipated to increase at an annual rate of 8.9% between 2022 and 2030. Elevating the consumer's awareness to wellbeing is the main driving factor for dietary supplements, which also necessitates the use of enzymes. Nutraceuticals market size was reported at $454.5 billion during 2021 and is predicted to rise at a compound yearly growth rate of 9% between 2021 and 2030. The worldwide detergent market was conveyed to be $133 billion during 2016. In addition, the global bromelain market size was reported at $24.2 million during 2021 and is predicted to rise at a compound annual growth rate of 7.1% between 2022 and 2030 because it is used in the healthcare, seafood processing, and dietary supplements sectors. The global market of functional foods was reported at $161.49 billion during 2018 as the need for fortifying food additives is growing. All of these industrial sectors apply the use of enzymes in their manufacturing steps.[120] Novozymes, BASF, DuPont, and DSM are considered the main enzyme manufacturers that can produce around 75% of the overall industrial enzymes.[118] North America and Europe made a huge profit estimated at 65%, while the Asia-Pacific market share was 30% of the revenue generated.[121] This revenue is expected to increase, especially with the wide applications of enzymes and the continuous search for new enzyme technologies.

7. EFFECT OF CORONAVIRUSES ON GLOBAL ENZYME PRODUCTION

The COVID-19 pandemic is by far the utmost alarming global health emergency that took place during this century.[122] Besides impacting global health, the COVID-19 pandemic possessed numerous adverse consequences on the economic, social, mental/psychological, environmental, cultural, and political aspects.[123] The growth of the enzyme market depends on the demand for the enzymes' end-uses, e.g., cleaning, biofuel, food/beverage, pharmacy, animal feed, etc.[124,125] Even though the industrial enzyme market witnessed a significant evolution in terms of its revenue during the past decade, it was dramatically affected adversely in 2020 due to the coronavirus pandemic. It was reported that, during the 2020 pandemic

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year, the highest enzyme market share was that of the food and beverage sectors, as well as the house care products and cleansers.[125] The demand for enzymes for industrial applications dropped severely during the last two years because of disruptions in global supply chains.[124] However, most of the pioneering companies in their fields tried to return their production intensity back and strengthen their production in other relevant products (e.g., sanitizers and handwashes). The influence of the COVID-19 pandemic was devastating, with the market perceiving a negative need throughout the world during the pandemic. The global enzyme market was significantly increased from an amount of $7.70 billion recorded during 2020 up to an amount of $8.9 billion during 2021; these values were recorded at a compound yearly growth rate of 14%.[125] A report comparing the global enzyme market between 2019 and 2020 displayed that a 1% decline in the enzyme market size was reached in 2020.[125] The market is continuously growing and is estimated to reach up to $13.25 billion during 2025 with a yearly growth rate reported at 14%.[125,126] Hence, the consuming rate is anticipated to continue increasing significantly until 2027 to reach up to $17.7 billion; however, this estimation could be disturbed again in the event of upcoming waves of coronaviruses.[124,126]

8. CONCLUSIONS

The growing technological developments of protein extraction together with novel advancements in protein engineering have led to proficient manufacturing of certain enzymes with enhanced characteristics. The demand for biocatalysts is steadily increasing in many industrial sectors. Several advantages of enzymes over chemical catalysts make them favorable alternatives for almost all industrial applications. Enzyme immobilization has several economic, ecological, and technical advantages, including operational stability and reusability. Enzyme immobilization is extensively applied in various industrial sectors, e.g., food, pharmaceutical, animal feed, textile, medical, and detergent sectors in addition to bioremediation/water remediation. Several enzymes are immobilized using different strategies (e.g., covalent binding and entrapment) on novel carriers and used in various large-scale processes. There still remains room for developing novel immobilization strategies.

The key drivers of the global enzyme market mainly involve the high demand from the manufacturers of detergents, pharmaceutics, textiles, animal feeds, biofuels, and cosmetics for these environmentally friendly substitutes. Although the industrial enzyme market witnessed significant growth and revenue during the past decade, it was adversely affected in 2020 due to the coronavirus pandemic. The demand for enzymes for industrial applications dropped severely during the last two years because of disruptions in global supply chains. Enzymes in their immobilized forms will obviously be more commonly applied in the near future.

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