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Recent trends in industrial resource manufacturing processes using biocatalysts

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Abstract

Enzymes promote various biological reactions necessary for sustaining human life by reducing activation energy required for reactions without causing permanent changes. Both plants and animals enzymes cannot be exploited for industrial application but microbial enzymes offers advantages such as easy to handle, fast multiplication and easy genetic transformation in specified conditions, easily immobilized, and high production yield with consistent quality. Microbial enzymes are produced by fermentation (submerged and solid state), worked for various bioprocesses through the inputs of rDNA technology, meta-genomics and with protein engineering. These enzymes play crucial functions in different array of industrial resources and form the backbone for a large number of industrial productions. In many cases, free forms of microbial enzymes have demonstrated the efficient bio-industrial application. These free forms of microbial enzymes have to be utilized in highly controlled environments whereas immobilized forms of enzymes show greater resistance to environment variations and recover in easy manner in comparison to free forms. Nowadays, microbial enzymes are applied in many industrial resources such as medical industries, food industries, detergent, textile, wastewater treatment plants, pharmaceuticals industries, etc. As per the definite applications, a preferred enzyme immobilization technique and appropriate carriers are selected. These techniques may be covalent binding, adsorption, entrapment, encapsulation, etc. This article mainly inculdes microbial enzymes, especially β-mannanase, Endo-β-glucanase (cellulase), Lipase, Protease, Amylase, immobilization techniques and utility in various industrial applications. Also briefly includes the biotechnological applications of xylanase, pectinase and keratinase. Furthermore, discuss problems and limitations related to industrial enzymes and market scenarios.

Keywords: Microbial enzymes, biocatalyst, immobilization, bio-industrial application

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Introduction

The development of today's enzyme industry we have is the consequence of fast development over the past few decades. In ancient times, enzymes that were available in the nature have been heavily used in food products productions like cheese, beer, sourdough, wine, leather, vinegar, indigo and linen were dependent on enzymes that were produced rigorously through spontaneous growth of microorganisms or enzymes available in improved added preparations. Over the past four decades, fermentation process made it easy to produce enzymes as highly purified and characterized products on larger scale. Since then the progress entered in the genera of enzymology into applied industrial value products with improved scientific process. The developments of directed evolution and protein engineering have more revolutionized the enzyme industry. These techniques offer to provide enzymes with improved activities and adjusted to new optimized conditions further allowing in expansion of bio-industrial applications. Recent day's developments in enzyme industry are the demand for sustainable process with highly augmented applications as biocatalyst in various industries [1]. According to Krik, et al (2002), the highly improved and efficient manufacturing of certain enzymes to an analytical purity grade was credited to the increasing new technological development in protein preparation, extraction and purification along with definite development in protein engineering techniques [2]. Even though, the growing technological development has created the useful and prominent enzymes, their bio industrial applications are hindered due to short shelf life, poor stability, sensitivity towards many techniques and environmental conditions [3]. In-order to fix the problem, advance enzyme immobilization techniques has led to the manufacture of targeted enzymes that are stable and robust [4]. These strategies have also enabled the recovery rate along with reusability of those enzymes which also improved the control of industrial process. Many of the disadvantages could be stopped and enhanced the enzyme production process by immobilization techniques [5]. Most of the industrial enzymes currently used are hydrolytic and applied for

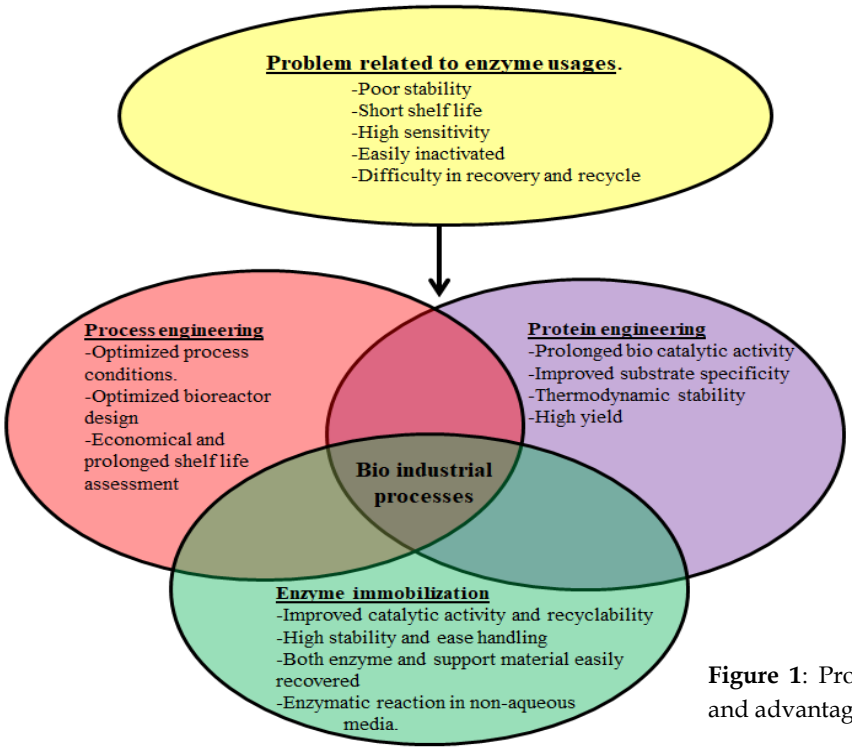

Figure 1: Problem associated to enzyme usages and advantages of bio catalytic processes.

the degradation of existing natural sources. In detergent and dairy industries, proteases are the dominant enzyme. Amylase and cellulose are the next industrially used enzymes such as the baking, starch, detergent and textile sectors. Usages of such enzymes in wide range industrial sectors is increasing rapidly in paper industries [6], chemical manufacturing [2], cosmetics [7], detergent [4] health care [8], dairy [9], baking [10], beverages (beer, fruit pulp and concentrate, wine industries) [2], and starch transformation industries [11]. Now a days biosensor manufacturing industries are using enzymes with wide specificity towards their biomarkers and also constantly been practiced in biofuel production and waste water treatment industries [4]. **Figure 1** describes the problem related to enzyme usages and advantages of bio catalytic processes.

This review will discuss the technical use of enzymes and technological advances, in particular, immobilization strategies which facilitated these developments along with problems and limitations of industrial enzymes and market scenario.

Microbial enzymes for bio-industrial applications

β-mannanase

Generally, mannans originated from simple sugar unit mannose and include different types such as mannan, galacto- glucomannan, galactomannan and glucomannan,. They consist of linear backbone of β-1-4-bonds of mannose sugar residues. The process of full decomposition and changes of mannans mainly involve exo-1-4-β-mannanase, endo-1-4-β-mannanase, and β-mannosidase [12] [13]. The plant cell wall heteromannans and mannans as the main part of hemicellulose fractions. The mannan-endo-1,4-β-mannosidase or 1,4-β-D-mannan mannohydrolase (EC 3.2.1.78) catalyses the decomposition of β-1,4-glycosidic bond to produce mannooligosaccharides of mannan, galactomannan, glucomannan and galactoglucomannan [14]. Particularly, mannan and mannooligosaccharides have drawn interest of food and pharmaceuticals industries due to various beneficial applications on human wellness. These oligosaccharides and poly-oligosaccharides are integral component of human wellness [13]. Mannanases have demonstrated various bio-industrial applications such as bio bleaching of soft wood pulps in the pulp and paper industries, enhancing the standard of food and feed, decreasing the thickening of coffee extracts, hydrolytic agents in detergent industry, oil drilling, slime control mechanism, pharmaceuticals applications, fish-feed additives [15]. β-mannanases isolated and produced form microorganisms is considered more feasible because of their high enzyme activity, high yield, low cost, and possess wide range environmental factors under control conditions. Those microorganisms primarily includes Philippine soil isolate *Bacillus subtilis* NM-39 [16], horse feces isolate *Bacillus amyloliquefaciens* CS47 [17], alkaliphilic *Bacillus sp*. N16-5 [18], *Bacillus sp*. MG-33 isolated from desert of Rajasthan (India) [19], *Paenibacillus illinoisensis* ZY-08 [20], acidophilic fungus *Bispora sp* MEY-1 [21], filamentous

fungus *Aspergillus niger* [22], marine bacterium *Vibrio sp.* Strain MA-138 [23], fungus *Sclerotium rolfsii* [24], *Streptomyces lividans* 66 [25], *Streptomyces thermolilacinus* [26], *Streptomyces sp*. S27 [27], our own lab grown *Bacillus sp*.CSB39 [28] and *Bacillus subtilis* subsp. *Inaquosorum* CSB31 [29], *B. subtilis* WY34 [30], *B. licheniformis* [31], etc. These microbial mannanases products are generally extracellular as well as inducible in their original states. Unfortunately, not all microbial mannanase are able to operate at any unfavorable conditions. Regmi, et al (2016) [28] has compared the different properties of various mannanase from different microorganisms resulting in different temperatures and pH stability and optimum conditions. This comparison indicates the production of extracellular and inducible enzyme forming stable mannanase can undergo the elevate production temperature and pH with greater shelf-life is suggested. Therefore, immobilization approach can be the best option for retaining the stable mannanase enzymes development.

Endo- β-glucanase (cellulase)

1,4 glycosidic bonds in cellulose polymers is hydrolysed by cellulase in-order to release glucose units. Cellulose considered as the most available and renewable bio-polymer obtained in waste products from agriculture [32]. These renewable resources relays on microbial hydrolysis of lignocellulosic waste and fermentation, hence appropriate pre-treatments are required to disassociate lignin structural formation and to increase easy accessibility in order to catalyze the raction-rate of biodegradation [33]. Mainly, cellulases are developed from microorganism such as bacteria and fungi and the cellulosic reaction occurred is due to catalyzes of the produced cellulose enzymes. Lignocellulosic waste and downstream process by microbial degradation of the produced reducing sugars and efficient decompose of cellulose into glucose, cello-oligosaccharides and cellobiose requires the action of different enzymes such as endo β-1,4-glucanase(EG; EC 3.2.2.4, randomly cutting internal bonds), β-D-glucosidase (BG; EC 3.2.1.21, decomposing glucosyl parts through cello-oligosaccharides and cello-biohydrolase (CBH; EC 3.2.1.91, decomposing units such as cellobiosyl from non-reducing sites) [34]. Recent microbial endoglucanase studies was produced by *Bacillus subtilis subsp. inaquosorum* CBS31 [35], *Bacillus licheniformis* C108 [36], *Bacillus sp.* CSB55 [37]. Their studies demonstrated as an efficient bio-catalyst for industrial application that enzyme catalyzed decomposition are operated and their results verify the change of the initial reactant to the final resulting product is spontaneous. They possess increasing hydrolysis and the greater applicability of the enzyme reaction. Wide ranges of cellulases that exist are different both spontaneously and structurally making them suitable for bioindustrial applications. Such practice is carried out in very undesirable and non-feasible color extraction from different pulps and fruit juices [38]. Some of them plays great role in the cleaning sectors as color brightener and fabric softeners. In few cases, these enzymes are also deployed in penetration of biomass in order to improve the nutritive value of food items and treating industrial waste [38]. Now a days these enzymes have developed their utility in pharmaceutical, animal feed, textile, paper industries, etc [39]. Having all these wide range applications and usefulness in industries, cellulase enzymes possess low stable characteristics, shorter shelf-life and high sensitivity and resistance [39]. Enzyme immobilization approaches of cellulase can highly reduce these drawbacks to be used in industries.

Protease

Proteases are the most applicable enzymes for biotechnological applications due to their global market. Proteases are the integral part of the biochemical processes that happens not only in animals, humans, and plants but also in some microbes. Currently, microbial proteases enzymes are commonly applied in food and feed industries, cosmetology, textile sectors, medicine and pharmaceutical setups [40]. On the basis of the peptide bonds breakdown, proteases are basically classified into acid, alkaline and neutral proteases. They are produced, purified and characterized from various living creatures showing wide substrate specificity and stable catalytic efficiencies [41]. As per Enzyme Commission, Proteases are hydrolases (group 3), that breakdown peptide bonds (sub group 4). On the basis of N-terminal or C-terminal peptide bonds cleave, they have been classified as exopeptidases and breakdown of internal peptide bonds as endopeptidases. Endopeptidases are more commercial than exopeptidases on the basis of bioindustrial applications [42]. Based on breakdown action (proteolytic), proteases are classified into cysteine proteases, serine proteases, aspartic proteases, threonine proteases, glutamic acid proteases and metallo-proteases. These enzymes can work on specific modification sites of proteins via cleavages such as activation of blood clotting, zymogenic enzymes, and also through activating of secretory protein throughout the membrane layers [41]. Now days, examination of proteases successful on catalyzing

various reactions in low temperature water have expanded its application for detergent use at room temperature. Though having wide applications in biotechnology, uses has been limited due to short lives, thus immobilization can be a turning point for enzyme stabilization and expand its application in different industrial sectors.

Amylase

Amylases catalyze hydrolyzing starch in sugars. These contain glycosidic hydrolases acting on α-1, 4-glycosidic linkages. The 3-D orientations possessed by amylases bear the linkage between substrates which support the breakage of α-1, 4-glycosidic bonds. Microbial amylases possess a wide spectrum of applications because of their higher stable raction than those directly derived from plants or animals sources. Currently, various microbial amylases are commercially available and have been successfully used in the wide range of industry such as food industries, detergent plants, fermentation setup, textile industries, and pharmaceutical industries [43]. The production of amylases is done by the application of either submerged fermentation or solid-state fermentation. These developing techniques depend on selected specified physico-chemical environments. The major consumers of amylase in industry are detergent sectors which comprises about one-fourth of total amylase market. According to Lahmar et al., 2017, in about 90% of the liquid detergent formulation, amylases are added for high effectiveness [44]. α-amylase that are used in cleaning starch-based stains such as cereals, fruits, sauces and vegetables from various fabrics are detergent additives. Among many, few amylases demonstrated good activity under certain extreme environmental conditions. For instance, α-amylase derived from *Bacillus cereus* GA6 isolate showed high activity at pH 10 and lower temperature ranges [45], *Zunongwangia profunda* [46], and *Bacillus subtilis* N8 [47] were experimented for detergent compatibility. There are amaylase that are available globally which are known for their efficient activities and applications. Stainzyme developed by Novozymes is one of the first amylase that shows significant activities at a varying range of temperature which is used in detergents. Similarly other amylases with good activities are Stainzymeplus which is considered as bleach tolerant and cold active amylase and the next one amylase named Preferenz S100 that is highly active at 16°C used by DuPont Industrial Biosciences for detergent applications. Microbial amylases are better adapted to extreme conditions in detergent industries. Various microbial amylases used in industrial applications include starch producing for the high efficient hydrolysis of starch-to-starch along with syrup of glucose-fructose. In most of the cases, amylases can be primarily and selectively applicable for the production and refinement of fine chemicals [48]. Preparation of digestive aids and starch syrups are widely used in food processing industries [43]. To maximize the application and stability of amylase, immobilization can be a useful tool.

Other enzymes

Xylanase is a family of multi-element enzymes which contains endo-xylanase, exo-xylanase , and β-xylosidases. Enzymatic hydrolysis of xylanase has showed various applications in waste treatment, food, textile, animal feed, ethanol production and pulp & paper industries [49] Xylanases as an accessory enzyme has demonstrated the lowering of enzymes dosage but it acts to remain a susbtrate-dependent reaction [50]. Low severe pre-treatments of lignocellulosic biomass are mostly used as hemicellulose characterization. Studies of synergism between cellulases and xylanases that take part to pretreatments severities ultimately increasing both glucose and xylose products [51] Similar studies were done from *Streptomyces sps* [52, 53]. Generally, keratin is a fibrous-structural polypeptide which is abundantly available beside chitin and cellulose [54]. Keratinase contains distinctive characteristics that claim its uses in bio-processing applications in bioconversion of keratin into valuable end products, treatment of textiles, leather depilation, and other various industrial uses [55] [56]. In comparison to hydrolysates obtained from other sources, keratinase has the greater tendency to penetrate the cuticles of nail and hair verifying it's increasingly uses in the cosmetic itemss formulation like skin moisturizers and hair conditioners [57]. Wheat protein, collagen hydrolysates and wool keratin have found their uses in the manufacturing of hair-care and skin-care cosmetic products [58]. One study shows that keratinase isolated from *Meiothermus taiwanensis* WR-220 which was immobilized on modified bagasse cellulose is used in the final treatment of industrial wastewater demonstrated effective decolorization of molasses from 84.7 to 90.2% which shows better result than the performance of other commercially available immobilized enzymes [59]. Pectinases are extensively used in fruit juice extraction and clarification in many food industries [60]. These days pectinases have shown application in various bio-industrial fields namely textile industries, fiber processing unit (plant based), oil extraction plants, treatment of industrial effluent and tea & coffee

processing units. There are evidences that showed pectinase application as an effective enzyme for whitening and great absorbance of the fabrics through bio scouring of cotton [61]. In this part of review we will discuss the application of microbial pectinase and its ease of handling. The fruit and vegetable processing industry uses pectinase for the lowering of thickness by enhancing the transperancy of juice as well as maceration of fruits and vegetables through the lowering of fermentation operating time [62]. In many bio-industrial processes, pectinase have demonstrated the vital features like the curing of coffee items, cocoa products, and tobacco leaves, canning of orange ingredients, and manufacturing of sugar from date fruits through refinement of vegetable fibers in starch producing steps [63]. Addition of pectinase during fruit crushing in winemaking aids the production of free flowing juice during the fruit pressing time that finally improves the filtration process and enhances the juice clarity which ultimately enhances the color and stability of red wines [64]. Other applications of pectinase are seen in pulp and paper industries, industrial waste water treatment, coffee and tea fermentation factories and oil extraction [65]. Research has shown that pectinases are promising option for next-generation prebiotics. Researches have shown that methylated pectin fermented by intestinal bacteria lead to the production of acetate, butyrate and propionate that are essential to human wellness. By enhancing the antioxidant potentials of diets, pectinases are employed to produce functional food components and nutraceutical products. [66].

Hindrance and limitations of bio industrially applicable enzymes.

Bearing the huge advantages in comparison to traditional catalysts, few issues hinder the utilization of industrial enzymes in their raw states. Enzymes have been used with widespread applications and most of them have been hindered by a number of limitations such as often-seen limited thermo stability, little substrate scope, low stero-regioselectivity elevated temperature, and extreme pH. Besides all these issues several technical and operational problems encounter for the applicability of enzymes in industrial settings that makes them realistically unreliable and difficult to handle with 100% ease for quality products. According to Bardy D. et al, 2009, several types of enzymes perform better in homogeneous catalysis systems when dissolved in water unlike the conventional heterogeneous chemical catalysts [67]. These various problems can be prevented or reduced with directed evolution by addressing and solving the problems with recent developed methodology such as protein engineering. The Darwinian concept to asymmetric catalysis has demonstrated a number of bio-industrial applications. Mutasysnthesis, metabolic pathway engineering, and fermentation techniques enrich the enzyme chemistry. These developed enzymes might possess the problems like weak stability, fewer shelf-life, high sensitivity to operational processes, easily inactivated by several mechanisms and trouble in recovery process, reusability as well as recycle. These various problems can be addressed by immobilization techniques making enzymes high stable and ease in handling, both enzyme and support material easily recovered, enzymatic action can occur in non-aqueous media, and improved catalytic activity and recyclability.

Immobilized enzymes in industrial processes.

Enzyme immobilization techniques entail entrapping or attaching enzymes within support materials. The major role of the solid support material is to make stable structure of enzyme and save their efficacy to greater periphery by exhibiting them more resistant to their provided process environment. This technique provides an easy option of recovery of enzyme and support materials and has been useful in medical, food, and pharmaceutical applications. Immobilized enzymes have shown much stablility and easy to control in comparison to free forms. Their enzymatic can happen in a non-aqueous where support materials store enzyme integrity making them even stronger. Moreover, the catalysts converted from homogenous states to different heterogeneous states demonstrate efficient enzymatic linkage [68]. Reversible methods of immobilization techniques include ionic bonding, adsorption, and affinity bonding whereas non-reversible methods of immobilization techniques include covalent binding method, entrapment technique, encapsulation process, and cross-linking.

Recently, because of wide industrial applications, enzymes have shown their application to catalyze various chemical reactions in major industrial steps [69]. These days enzyme development and protein engineering have shown potent characteristic of bio catalytic processes where large number of molecules were synthesized using various enzymes. Such enzymes are very useful in industrial bioprocesses incorporated with immobilization techniques. Their benefits and scope of applications can be improved with protein engineering, facilitating to eco-friendly process from chemical one [70]. The implementation of engineered

transaminase for the production of sitagliptin is an excellent example of protein engineering and immobilization technique to develop more stable and catalytically efficient enzyme [71]. Similarly modified lipases have extended their selectivity of acrylate synthesis [72] and cocoa butter analogs for the synthesis of 1,3-triglycerides [73].

The decision of utilizing enzyme in insoluble form or free soluble is primarily depends on the cost and application of the specific enzyme. Insoluble forms are a specialized type of heterogeneous catalysis where their recovery and subsequent reuse happens by maintaining activities over a longer time period ultimately showing various applications to a wide range of process methods [74]. In industrial settings, simplicity and cost-effectiveness are prioritized when it comes to enzyme immobilization methods. Currently, the most commonly employed techniques for immobilization involve physical methods (adsorption or physical entrapment), as well as chemical methods (covalent binding and cross-linking). As mentioned earlier in this review, most of the industrial process preferred simplicity and cost effectiveness when it comes to enzyme immobilization methods. Now a day, the most used techniques for enzyme immobilization includes adsorption or physical entrapment and covalent binding and cross-linking. Generally, enzymes are used in detergent, textile, medical, pharmaceutical, food, feed and waste water treatment processing industries. Figure 2 shows the schematic illustration of recent industrial enzyme trends.

Food industry

The demands of enzyme in any food industries are to develop huge amount of cost-effective products. To be beneficial and cost sensitive products, most food industries need to have the cost of biocatalyst to be low, stable for a maximum number of cycles to be carried and continuous flow operational settings over batch processes are preferred. Certain types of cost effective as well as high stable enzymes are extensively used in large-scale continuous processes for the development of a wide range of food products. As for examples, their application in the development of high fructose corn syrup, a popular sweetener found in beverages and various food items has deployed, or applied direct as a food component. D-Glucose/xylose isomerase utilizing D-xylose, the native substrate that possess wide substrate specificity along with efficient catalytic properties that can efficiently converts D-glucose to D-fructose in bio industrial application. [75, 76, 77, 78]. Allulose- "zero-calorie" sweetener which is considered sweetness similar to dextrose. CJ Cheil Jedang Corp in 2011 had demonstrated that the ketose – 3 - epimerases (EC 5.1.3.31) expressed in numerous microorganisms possesses the properties of interconvertable fructose and allulose [79, 80, 81]. Another emerging sweetener in foodstuffs is tagotose [82]. Galacto-oligosaccharides as sysnthesis of oligosaccharides have received many health benefits and braod range of application as prebiotics. Galacto-oligosaccharides can be produced from B-galactosidase (EC 3.2.1.23) through consective translactosylation reactions with a terminal glucose units. [83, 84, 85, 86]. Lipase CalB for vitamin C esters from Lipase B from Candida Antarctica [87]. Triglycerides from Lipase from Thermomyces lanuginosus and Lipase from Rhizomucor miehei application as cocoa butter equivalents through enzymatic transesterification is used for commercial manufactured of different oils and fats with targeted physical properties through removal of hydrogenated trans-fats which is responsible for serious health issues [88, 89]. Omega-3 fatty acids, generally sourced from marine algal and fish oil, are taken from a process involving the direct esterification of free fatty acids in fish oil with ethanol. This process of esterification mostly utilizes immobilized CalB (Lipase enzyme) and is followed by distillation process to separate the ethyl ester fractions. The obtained fractions are enriched in eicosapentaenoic acid (C20:5), while the remaining free fatty acid fraction is enriched in docosahexaenoic acid (C22:6) from Lipase B from Candida Antarctica [90 ,[91]. Beta-galactosidase as hydrolysed lactose in lactose-free dairy products which have similar function to the human intestinal flora that hydrolyses in galactose and glucose. Now a days, industries have developed two hydrolytic processes using beta-galactosidase that hydrolyses lactose into glucose and galactose (monomers) and this has been applied to remove lactose from milk [92, 93].

Detergent industry

Detergent's formulation for the removal of strong stains enzymes have long been used those ordinary chemicals cannot act. Small amount of detergents based on enzyme formulation are able to remove stains at ambient temperatures. For example, amylases in detergent formulation aids in remove of stains like curry gravies, potatoes, cereals, chocolates and other starch based stains. In the same way, stains of blood, egg, meat, fish, grass and human sweat stains are easily removed with proteases action formulated in detergents. Similarly, lipases are effective against removal of oil and fat stains. Cellulases formulation support in improving the color brightening of cotton-related fabrics and softening cotton

final products textures [94]. Improved properties of immobilized enzymes not only enhance cleaning activities but also preserve the enzyme's catalytic activities, and resulting in no harm on wool based fabric. Soares, J. C, et al. 2011 investigated the proteases that breakdown the silk and wool keratins (natural protein fibers) leading to irreversible damages to the fabrics' quality [95], whereas immobilized protease that are covalently bonded with eudragit on wool fabric retain the more than double the original tensile strength compared to free enzyme (37%). Also reported enzymes that are immobilized showed no harms on the quality of wool fabrics [96]. Second to protease comes lipase as the most important detergent enzyme. Lipases formulated for laundry, dishwashing and cleaning detergents used in harsh oil stain removal works under wide temperature range and pH ranges. In normal water, free form of lipases possesses little cleaning effectiveness because of mass transfer barriers within the enzyme and oil from stains; therefore lipases were immobilized using glutaraldehyde which resulted excellent oil removal ability by preserving catalytic efficiency in washing cycles by more than 80%. [97]. In another case, lipases were immobilized using arylamine glass beads resulting to effective removal of oil stains form cotton fabrics and has the ability to be used for 100 wash cycles [98]. These reported cases showed that the immobilized enzymes possess more catalytic activity and stable results in bio industrial applications.

Pharmaceutical industry

To produce the enzymes that meet the demands of fast-paced and high-volume of industries for valuable molecules production is a significant challenge to traditional way of chemical synthesis. In order to slove these issues, it is necessary to address the problems through the application of protein engineering and immobilization techniques. By the application of these techniques, industries can develop enzymes that are capable of producing pharmaceutical grade chiral molecules efficiently. In general, Lipases developed from lipase B of *Candida antarctica* (EC 3.1.1.3) are the widely applied catalysts for the productions various valuable molecules such as cosmetic additives in personal care products, active pharmaceutical formulation, food ingredient composition due to their wide selectivity (region-, chemo-and enantio-selectivity). In bio industrial process, immobilized form results wide ranges of applications due to its high tolerance to organic solvent, temperature ranges and broad selectivity. Lipase B from *Candida antarctica* (CalB) immobilization enabled 99.9% reduction in cost in comparison with Novozym 435, which is commercially available as CalB immobilized on a divinylbenzene/methacrylate resin. In contrast to this, Martell M. has reported immobilizing CalB on an octadecyl activated methacrylate resin demonstrated into high stability with huge increase in activity, which was due to best possible interaction of the hydrophobic octadecyl group with lipases [99]. Now a day, commercially Sofosbuvir for lipase B from *Candida antarctica* is used in treating hepatitis C capable of overcoming the high mutagenicity of HCV and presence of various genotypes and subtypes [100]. The patent from Gileadhas utilizes immobilized CalB during enantioselective hydrolysis process, forming an acetate ester into chiral alcohol. This particular step was used for the synthesis of Sofosbuvir involving multiple chemical process steps [101]. In the same way, industrial production of Penicillin G amidase for β-lactam antibiotics involve two different pepicillin G acylases engineered for the hydrolysis of benzyl-penicillin to form the β-lactam nucleus 6-APA and the another one for the development of semi-synthetic β-lactams (ampicillin or amoxicillin) [102]. Lastly, immobilization of transaminases applied in the bio industrial production of APIs demonestrates the first notable result of using other enzymes besides the hydrolases series. This was achieved by using a combinatorial model and direct evolution leading to highly active and more stable R-selective amine transaminase in the production of sitagliptin for the treatment of diabetes [103].

Medical device industry

Patients with cystic fibrosis use lipase in medical device (pancreatic enzyme replacement therapy) in conjunction with meal to increase the absorption of fat and other nutrients. These available combinations contain triglycerides but fatty acids due to the weak and low range stability of hydrolyzed fats [104]. These devices not only increase life expectancy of the patients but also help to improve fat absorption resulting into improving chronic lung disease along with improving cognitive ability with reduced time duration needed for parenteral nutrition [105]. Similarly, use of urease is highly specific enzyme in dialysis system of an artificial kidney setup to remove the urea in renal failure patients. Urease catalyzes the hydrolysis of urea into ammonium and carbon dioxide [106]. Bio industrial applications of the urease are wide, from the hydrolysis and removal of urea in waste water treatment, beverages settings, foods processing and more confoundedly the removal of urea from blood for extracorporeal detoxification as well as in

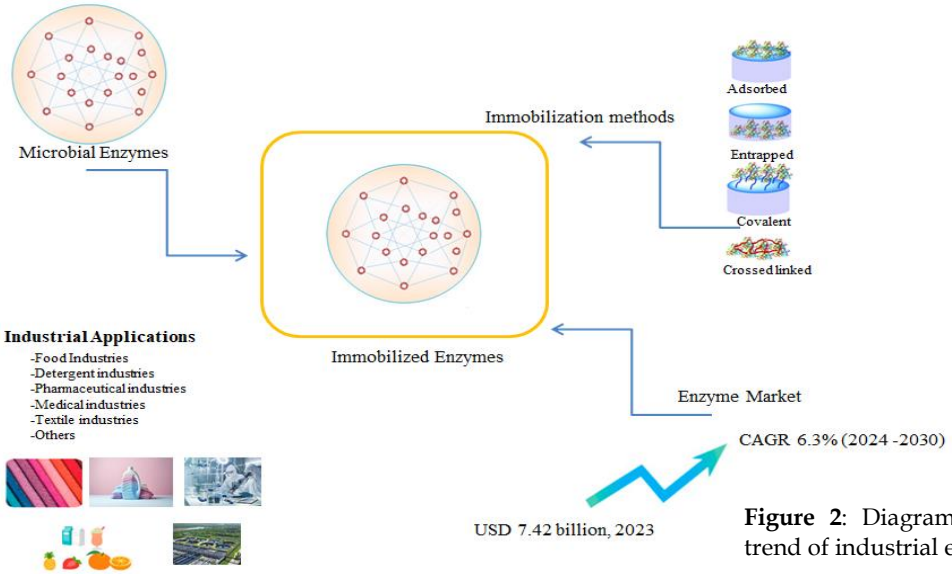

Figure 2: Diagrammatic representation of trend of industrial enzyme.

regeneration system of dialysate in transplant kidneys. The use of immobilized urease is incorporated in a portable dialysis machines to catalyzes the hydrolysis of urea to ammonium and carbon dioxide, which is based on sorbet regenerative dialysis system [107]. Now a days, in medicine and clinical diagnostic, enzyme-based biosensors have wide an application of immobilized enzymes [108, 109], agriculture industries [110], environmental monitoring [111], food [112], and also in food safety [113]. Due to these high sensitivity and specificity of the catalytically active enzymes towards their target areas and molecules claimed their benefits of these technologies and techniques.

Other industries

A good number of enzymes used in textile industry have been noticed recently. As for example keratinase enzyme was immobilized with chitosan-β-cyclodextrin which demonstrated high thermal stability and preservation stability was greatly enhanced in comparison to the free form [114]. Cellulases are also used in textile processing even though poor recovery and reuse of the products. This is due to the capacity to modify cellulosic fibers and generating high quality textiles. Immobilization of cellulase has provided an excellent pilling resistance towards the fabric with more tensile, that gave a more recovery as well as reuse of the cellulases for few successive operation cycles [115]. Similarly, minimum decreasing of the tensile force and higher quality of whiteness were achieved [116], and epoxy resin with immobilized cellulose was incorporated in bioploishing of fabric in few effective cycles with no tensile strength reduction [117]. In general, the wool producing companies uses harsh chlorination technique to finally get shrink resistant fabric final products [95]. A research carried by Smith, E et al, 2010 demonstrated that use of proteases in place of chlorination resulted into efficient shrink resistance products without harming any environmental conditions in contrast to chlorination [118]. These days, immobilization technique is highly regarded an excellent alternative for wool shrink-resistant fabric and could replace the standard chlorination procedures [119]. Untreated waste water form industries are causing a very big problem to the world. Disposal of phenolic sand dye have raised huge concerns form scientific communities because of their high carcinogenic and mutagenic properties, poor biodegradability plus toxicity. To solve this issue, various enzymes are used such as laccase, preoxidases, and azo reductases to clean the dyes at the time of water treatment procedures [120]. Several enzymes have the limitation of decreasing their enzyme activity and not clearing dye easily. Different immobilization process with laccase [121], and magnesium peroxidase [122] are used to reduce the color of effluent water. Similarly, phenolic compounds from industries' untreated waste water are major water pollutants bearing high toxicity and very low biodegradability properties. Basically, use of high efficient and ecofriendly immobilized enzymes is a better option for extracting and processing phenolics from wastewater pollutants [123]. Various oxidoreductases, including laccase [124, 125], tyrosinase [126, 127], and horseradish peroxidase [128, 129, 130, 131] were immobilized to achieve higher catalytic activity, more selectivity, and efficient thermal stability for the removal of phenolic compounds.

Industrial enzyme market size and trend.

In 2023, the value of global industrial enzyme market was estimated at USD 7.42 billion and is projected to rise at a

compound annual growth rate (CAGR) of 6.3% from 2024 to 2030. Future of global enzyme market scale is anticipated to fuel the growth by increasing demand for bakery products, fruits juices, detergent products, textiles and animal feeds. The food and beverages sectors account the highest revenue share of 21.05% in 2023. Now days both the free form and immobilized form of enzymes are extensively used in the production of various food and beverages items (including cheese, vegetables & fruits, oils & fats, grains), also in other food processing sectors such as baking, brewing, and dairy. These days' food and beverages sector primarily use proteases, lipases, and carbohydrate. Also enzymes like proteases, lipases, and amylases have found their wide application in the formulation of detergent products. In particular, proteases are commonly used in detergent products to efficiently remove protein–based stains such as grass, eggs, sweat, and blood. In the similar way, amylases find their application to remove stain developed by starchy food products (chocolate, custards, gravies, oatmeal porridge, mashed potatoes, and spaghetti). Enzymes that are incorporated into dishwashing and laundry detergents products do not contain chlorine. As a result, the demand for global industrial enzyme markets in animal feed production is estimated to increase in order with the projected CAGR.

The COVID-19 pandemic has significantly impacted on various sectors worldwide, causing disruptions in supply-demand chain. The global enzyme market also experienced a slight decline in demand-supply gap for products used in biofuel and textiles as a result of shutdown of the automotive industry. The crises witnessed the overall economic slowdown. However, the enzyme markets as a whole witnesses a strong adoption of enzymes in animal feed products, food and cleaning items. The COVID-19 pandemic educated the importance of hygiene and cleanliness in human life which emphasized the need of enzyme-based cleaning products. As a result of consumers' awareness of their health and lifestyle choices, there is a significant shift towards healthier lifestyle with hygiene and cleanliness products. The shift to healthier lifestyle choice have a positive impact on the global industrial enzyme sectors as it increased the demand for enzymes in various applications that help to maintain healthier living with enzyme-based cleaning and hygienic products. Other factors enhancing the enzyme modern market constitute the high market demand from various detergent producers, textile producing industries, pharmaceuticals sectors, animal feed producing industries, biofuel industries, cosmetics manufactures to their eco-friendly and safe bio-industrial processing alternatives to regular ordinary methodology [132]. At present, North America holds the highest revenue share of enzyme market with 37.65% in 2023 followed by European Union while the increasing trends of enzyme market in Asia-Pacific bears the share of 30% of the total revenue generated in this sector [133].

Conclusions

Enzymes are proteinases in nature and are involved in catalyzing various biological and biochemical processes. The growing advancement of protein extraction and need for the development of more sustainable and cost effective process together with the rapid progress in protein engineering coupled with the immobilized enzyme technology presents a bright future for the use of bio catalysis in industrial settings. Enzymes provide essential and advantageous benefits to the industrial processes over orthodox chemical catalysts applications which not only make them favorable alternatives for almost all industrial applications but also provide efficient and durable enzyme solutions. Enzyme immobilization process has various benefits such as process simplification with ease handling, economic, lowered environmental impact, and technical benefits, covering operational constant stability and efficient reusability compared to chemical synthesis [134]. Advancement of novel and superior performing end products and rapid development in the technology would enable industrial enzyme manufactures to cash on the huge unseen potential in the market. In order to develop such prospects in all segments of bio industrial application, industrial enzyme manufactures are increasingly using the developed protein engineering technique alongside immobilization processes for developing other application-specific enzymes. Enzyme immobilization is hugely applied in various industrial segments like foods, feeds, pharmaceuticals, textiles, waste water treatment, medical devices, biosensors, detergents in addition to bioremediation or water remediation. Having all these benefits of immobilization techniques, there is still room for development new and novel immobilization strategies. Due to this, enzyme industries have been compelled to actively look into the development and enhancement of new and existing enzymes to use unconventional substrates aiming to broaden the range of applications in the sector of chemical molecules. However, overall industrial enzyme market saw a good demand during COVID-19 on books of increasing adoption of enzymes from various

industries including animal feed, food, and cleaning segments. With increasing consumer awareness on health and lifestyle, there is a huge shift in demand for healthier life style is expected to positively change in industrial enzymes sector.

Conflict of Interest

The author(s) declared that there is no potential conflicts of interest.

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