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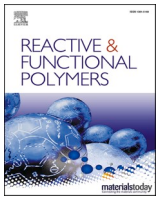

Review

Employment of polysaccharides in enzyme immobilization

Archita Sharma [a], Karan Singh Thatai [a], Tanya Kuthiala [a], Gursharan Singh [b], Shailendra Kumar Arya [a,*]

[a] Department of Biotechnology, University Institute of Engineering and Technology, Panjab University, Chandigarh, India
[b] Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara 144411, Punjab, India

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ABSTRACT

Analogous to free enzymes, immobilized enzymes are sturdy and resistant to environmental alterations. With unmatched high catalytic activity, turnover number, and selectivity, enzymes have the potential to utilize them as catalysts concerning practical applications which sometimes might get an obstacle due to the low thermal and chemical stability of enzymes. One of the best and out of numerous tested approaches, enzyme immobilization which makes use of varied enzyme-carrier interaction has made a remarkable dissimilarity. Another concerning concept, discussed here in this review, is the exploitation of polysaccharides which are carbohydrates made up of more than ten to thousands of monosaccharides linked through glycosidic bonds. Numerous natural polysaccharides (cellulose, chitin, alginate, starch, carrageenan, pectin, etc.) base supports have been utilized in the recent past by amalgamating diverse immobilization techniques such as adsorption, covalent binding, entrapment, encapsulation, affinity immobilization, etc. The call for polysaccharides all over the globe has increased in the past few years due to their elevated usage in diverse sectors as a resource for green and sustainable materials. Additionally, such base supports are easily available, have an easy fabrication process, insolubility in an aqueous environment, are biocompatible, non-toxic, biodegradable, and physiologically inert. Undoubtedly, considering highly effective, economical, and skilled biotechnological processes, it is, nowadays, regarded as a promising approach for monitoring environmental conditions, textile-based industries, biotransformation, pharmaceutics, diagnostics, and food industries. This review gives an insight into a brief background of an enzyme, the merits of the enzyme immobilization approach, and why the concept of exploitation of polysaccharides with enzyme immobilization is grabbing a lot of attention from researchers nowadays.

1. Introduction

The connection between people and enzymes has developed over time. In any event, where there was no understanding of enzymes or biocatalysts, old Egypt individuals created breweries by enzymatic aging. Following two or three millennium years, protein investigations have been advanced essentially. Proteins that accelerate numerous chemical and biochemical reactions are defined as enzymes. Enzymes are organic catalysts and are ubiquitous in vegetation, creatures, and microbes, where they accelerate crucial operations of life which ensures the survival of human beings. The developing information and methods to enhance the making and cleansing system of enzymes results in the development of numerous proteins at the diagnostic level for their respective biotechnological applications. Proteins are usually associated with a broad array of conventional forms of nourishment, for example, cheddar making, lager fermenting, and liquor manufacturing units. Later advancements in the field of biotechnology, particularly in the designing of enzymes have resulted in developments of a variety of proteins with improved characteristic features such as activity at ambient temperatures, enantioselectivity, regioselectivity, and better specificity towards substrates [1–3]. This has prompted the foundation of the latest, customized proteins for the latest uses, where proteins have not been utilized earlier [4].

The utilization of enzymes in various ventures is consistently expanding, particularly in the past two decades such as the extensive use of enzymes in (i) food industries which constitutes heating [5], dairy items [6,7], starch removal or alterations [8] and refreshment handling such as lager, wine, and products of the soil juices, (ii) textile industries where enzymes have discovered an extraordinary spot because of their impact on final results [9]. In other industrial applications such as mash

* Corresponding author.
E-mail address: skarya@pu.ac.in (S.K. Arya).

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and paper making [10] and cleansers [11], the utilization of enzymes has become an unavoidable raw material to prepare methodologies when an ideal finished result is generally required. The use of enzymes in progressively present-day businesses deals with biosensors which flourish quickly due to the significance of proteins in biosensors [12–14]. Furthermore, human services and pharmaceutic products like ibuprofen, naproxen, etc [15] and synthetic products [16] are progressively taking the point of interest which further bolsters the role of biocatalysts in industrial applications. In the recent decade, enzymes have been majorly exploited in different sources of bioenergy as well like bioethanol, biodiesel [17,18], biohydrogen, etc [19]. Additionally, the exploitation of enzymes can also be seen in managing and recycling the variety of wastes particularly solid wastes, and filtration of wastewaters thus advancing the lifestyle and environmental sustainability [20–22].

Enzymes have significant potential in contributing to less explored lipid technology bio-industries. They have been exploited for the *in-situ* metabolism of lipids and *ex-situ* diverse industrial applications [23,24]. In comparison to the aqueous solvents like methanol, acetone, DMSO (dimethyl sulfoxide), biocatalysis has been exploited significantly in a non-aqueous environment such as supercritical fluids (SCFs), ionic liquids (ILs), and deep eutectic solvents (DESs) for the development/modification of numerous lipophilic materials and raw materials [25–27]. Also, they have been used to synthesize structured lipids [28]. Structured lipids (SLs) are broadly defined as synthetic fats and oils with certain functional and pharmaceutical applications. These structured lipids consist of numerous steps namely hydrolysis, alcoholysis, glycerolysis, esterification, inter-esterification, and transesterification, respectively. The generated products can further be employed in various applications of food industries and pharmaceutics. The major issue revolves around the miscibility of lipids due to their varied nature and polarity. To overcome such issues, different solvents have been broadly exploited as reaction media [27]. Other important applications of enzymes constitute (i) recovery of cheese [29,30], (ii) enhancing the flavor and production of enzyme-modified cheese (EMC) [31], (iii) biodetergents [32–34], (iv) chemical catalysts [35,36]- the unification of biocatalysis and chemocatalysis renders the eco-friendly characteristic of biocatalysts, strong reactivity of chemical catalysts, reduced costs, and less generation of wastes, decreased operational time, easy handling of unstable intermediates and hence better selectivity and yield of the process [36], (v) agriculture products such as pesticides, insects, etc [37], and (vi) enhanced thermotolerance [38].

All the aforementioned desirable qualities of enzymes are frequently obstructed due to (i) less operational stability, (ii) high cost of production, (iii) moderate shelf-life, (iv) substrate and product inhibitions due to steric exclusion of the substrate from an inhibition site with interference with the active site of an enzyme [39] and thus cumbersome recovery and reusability [1,40–42]. Such downsides have been overcome by numerous strategies intending to enhance the biocatalytic process for improving the yield of products like directed evolution [43], rational enzyme design [44], enzymatic modifications [45], and enzyme immobilization [46]. Among all the aforementioned strategies, enzyme immobilization has been considered a promising technology for overcoming such limitations.

Presently, enzyme immobilization is an effective strategy for improving the stability, sturdiness, and reusability of the diverse enzymes even under harsh reaction conditions such as extreme pH, temperature, salts [47], and organic solvents which renders them an economical and practical approach when dealing with numerous industry-based applications [4]. The immobilization step will give partition and takes away all the impurities of enzymes and hence can be reused for the next cycle of reactions. The immobilization process has greater strength due to the presence of additional chemical and physical conditions and a simple enzyme transportation process from the starting to the next and hence makes the total procedure highly economical or financially stable [48].

Enzymes like carbohydrases, proteases, lipases, and oxidoreductases, are majorly utilized in food industries due to their magnificent catalytic activity. Nevertheless, free enzymes have poor stability concerning pH, heat, and temperature. Also, it is very much difficult to recover and reuse them, hence, it is extremely recommended for improving the stability and reusability of enzymes. Numerous techniques or approaches are intending to enhance the stability and reusability of enzymes namely enzyme immobilization, enzyme modification, protein engineering, etc. Among them, enzyme immobilization is periodically used for improving the characteristic features of enzymes [49].

In this manner, enzymes that have been immobilized are versatile to every sort of mechanical procedure. Besides, the immobilization of protein is valuable for chemoenzymatic cascade and multienzyme processes [50]. Enzymes play a significant role in manufacturing numerously biocompatible, biodegradable, and renewable resources with particular functions [51,52]. Nonetheless, when dealing with single enzymes, it is extremely difficult to perform complex catalytic reactions [53] whereas the cascade reactions are catalyzed by various enzymes and have an important role during the performance of complex reactions [54]. An intracellular catalytic system is defined as a multienzyme complex [55] where one enzyme is used as a substrate for the neighboring enzyme which further results in acceleration of the rate of reaction [56]. The intermediate enzymes render an effective transfer from one active center to another after substrate consumption [57]. Additionally, multienzymes catalysis decreases the loss of intermediate enzymes because of diffusion and results in the production of fewer by-products and hazardous pollutants [58]. However, integration of such multienzyme systems for accomplishing complex reactions has some serious implications [59] such as optimum operating conditions for every enzyme [60], single usage [61], and less stability [62]. Recovery of free-form multienzymes is laborious which results in higher operational costs and reduced production efficacy, limiting their exploitation in diverse industrial applications [63]. Also, certain multi-step reactions need to be catalyzed through a series of coupling system enzymes [64,65]. The suitable methods for immobilization of enzymes, loading efficacy, stereoscopic structure, and performance in various non-aqueous media, pH range, and protein structural rigidity are certain key features of such enzyme systems [66]. Therefore, all over the globe researchers are constructing synthetic cascade systems through the use of suitable functional enzymes [67].

The immobilized multienzymes offer fast conversions of chemical reactions, simpler downstream separation, more yield of pure products under mild reaction conditions [68,69]. Comparatively, the multienzyme co-immobilization is more effective due to (i) restriction of diffusion of unstable intermediates into the external environment [70], (ii) better stability, and (iii) improved efficacy of co-immobilized multienzymes [39]. Nonetheless, the co-immobilized multienzymes frequently often exist certain difficulties such as (i) formation of concrete reaction process from the substrate to the product in the cascade catalysis system [71], (ii) randomness [72], (iii) reduced catalytic efficiencies to free enzymes [73], (iv) high operational costs [74], and (v) diffusion barrier of the supports [75]. Hence, developing complete and effective artificial multienzyme coupling systems, molar ratios of enzymes, sequencing of immobilization process, and surface properties of the carrier are important to accomplish catalysis reaction [76].

The enzyme immobilization inside porous support has numerous protective effects on the structure of an enzyme under a varied environment which will ultimately protect the enzyme from getting distorted and hence no loss of activity [39]. Additionally, it is easy to separate generated product, it has easier reactor operation, better selectivity [77] due to alteration of geometry of the active center [41,49,78], stabilized conformation of enzymes [79], elimination of diffusion limitations by lowering the diffusion of substrate and therefore the less concentration of substrate will increase the activity of an enzyme [39,80]. The improvement in the characteristic features of immobilized enzymes is due to the (i) prevention of the dissociation of the subunit

from the multi-subunit immobilization approach [81,82], (ii) prevention from the aggregation of enzymes, (iii) autolysis/proteolysis by enzymes proteases [39,49], (iv) rigidification of the structure of enzymes through multipoint covalent attachment [83], and generation of desirable microenvironments [84].

The enzyme immobilization concept is correlated with the reduction in the activity of enzymes or other catalytic features. In the majority of the cases, enzyme immobilization results in the minor distortion of the structure and further results in the final characteristics of the enzymes [74]. Such distortion in the structure is significantly uncontrolled but creating a large biocatalytic library through the utilization of diverse immobilization approaches ensures a solution to improve the properties of enzymes [85]. Nevertheless, the improvements in the performance of enzymes are usually not associated with the production of a more active or selective molecule of an enzyme but it is due to certain artifacts which can change or hamper the activity and stability of an immobilized enzyme [39].

Multiple chains of monosaccharides are made out of rehashing singular entities or monomers of monosaccharides which are covalently bonded to one another through glycosidic bonding are termed polymeric carbohydrates [86]. The monosaccharides/glycans for the most part which consists of 3-9 carbon atoms and fluctuate in the stereochemical arrangement and the structural sizes at multiple carbon atoms. Polysaccharides made out of just a single sort of monosaccharide are depicted as homopolysaccharides [87]. Likewise, if at least two various types of monomers are available, then it is termed heteropolysaccharide [88,89]. The monosaccharides are found in the straight-chain structure which is an extended structure along with polysaccharides with branches and curves relying upon sort of monomeric units linked and the nature of the carbon which is linked to it [90]. The most widely recognized monosaccharides that show up as associated with glycans are fructose, glucose, mannose, and galactose [87]. Many polysaccharides are gathered from various sources, for instance, seaweed extracts, carrageenan, agar, alginate, cellulose, gelatin, locust bean gum, guar gum, the starch of tapioca and potato, gums like tragacanth and Arabic, gums of gellan and zanthan, shellfish, chitosan, chitin, carboxymethylcellulose (CMC) [91].

Polysaccharides are used as medicines/drugs as bonding agents, biological materials as drug carriers [92–94] because of various reasons, for example, (a) polysaccharides are abundantly present in the environment and the techniques for their disengagement from organic sources are well described and recorded [95], (b) minimum exploitation of eco-friendly polysaccharides-based biopolymers, [96], (c) functionalized polysaccharides via synthetic and protein-related techniques [97]. Glycans have a wide range of characteristics for improving numerous kinds of carrier systems [98], for instance, ionic glycans exhibit ionic sensitivity and pH which results in an enhanced catalytic response in the drug-carrier mechanism [99]. There are few applications of glycans in interpenetrated polymeric networks (IPN) and gel manufacturing that shows unexpected characteristics as compared to the large-sized molecules [99], and (d) classification of functional groups based on their availability and composition as compared to the other large-sized biomolecules such as peptides, enzymes, etc [100]. Such kind of qualities renders glycans a perfect biomaterial for manufacturing a suitable carrier mechanism for discharge the encapsulated drug at a particular location and time in an ongoing pharmaceutical reaction, reacting to the particular biological catalyst [99].

In general, there are various physical and chemical technologies for immobilization of enzymes on the solid carrier/transporter such as adsorption, entrapment, covalent binding, cross-linking, and encapsulation via van der waals forces, hydrogen (H) bonding, and ionic interactions based on nature of the carrier and final product. All these enzyme immobilization methods avoid any alterations in the local structure of the enzymes with efficient binding to the active sites of an enzyme from any disruption [101]. The characteristics of both carrier materials and enzymes have a significant impact on the manufacturing of enzyme support. The collective action between two ensures explicit biochemical, chemical, mechanical, and kinetic characteristic properties. Numerous materials have the potential to behave as carriers for an enzyme. The major concern revolves around the cost of the carrier, availability, stability, condition, and type of the reactors. Apart from that, certain physicochemical properties must be considered like particle size, pore structure, surface area, and types of attached functional groups [91].

Particularly, the carriers are categorized into organic and inorganic carriers, respectively. In the case of organic carriers, natural polysaccharides such as chitin, chitosan, pectin, cellulose, alginate, and few synthetic polymers are broadly exploited. Nonetheless, polysaccharides have grabbed significant attention of researchers from all over the globe for their utilization as enzyme carriers due to certain advantages such as easy chemical modification following the nature of immobilization, utilization in a non-aqueous milieu as native enzymes can only exhibit their activity in aqueous reaction environment, easy manipulation of enantioselectivity, chemo selectivity, regioselectivity properties of enzymes for specific application post enzyme immobilization process which aids in improving the yield of the final product, reverse an unfavorable reaction, and can control the homogeneity of a reaction. Apart from numerous benefits, organic solvents can harm the immobilized enzyme due to the desorption of enzymes from the polysaccharide-derived carriers/base support [91].

The adsorption method is fast, easy, and cost-effective with no use of chemical reactants. The disadvantage lies in the easy degradation of the enzyme due to desorption owing to the alteration in the pH, ionic strength, and concentration of the substrate. Supports like collagen, chitin, cellulose, polyethylene glycol (PEG) have been used for enzyme immobilization via the adsorption process. Another method covalent binding is firm and permanent but the issue is the frequent inactivation of enzymes thus hampering the catalytic activity. Concerning their applications in food industries, this method has restricted use due to the utilization of chemical reagents. In this method, silica, alumina, polyvinyl alcohol (PVA), bentonite, etc are being exploited as base support. The cross-linking method enhances the binding and stability of the immobilized enzyme but the immobilization procedure is time-consuming and utilizes expensive chemical reagents. It may also change the catalytic activity of the enzyme. Considering the entrapment and encapsulation methods, the advantage is that there is no requirement of modifying the enzyme chemically. The process is simple and is easy to scale up. But, the entrapment approach is not recommended for hydrolyzing high molecular weight (MW) polymers whereas, in the case of the encapsulation method, viscous solutions clog the reactor. For both the methods, gelatin, cellulose, nylon, PVA, calcium alginate are used as support for the immobilization of enzymes [101].

The characteristics of enzymes and transporter materials impact the manufacturing process of enzymes. The common activity mediating in these two characteristics leads to the formation of an immobilized enzyme obliging exact biosynthetic properties, kinetic and mechanical characteristics. There are different types of synthetic polymers, inorganic solids, and biopolymers that can be exploited for the arrangement of such immobilized enzymes. Besides, enzyme transporter has better physical strength, resistance against microbial contamination, regeneration ability, sturdy, and cheap [102]. The section recognizes the capability of polysaccharides in the region of protein immobilization and examines different guides to acquire information on this [87].

2. Enzyme immobilization

The enzyme, which is made of dynamic cells, is a proficient biocatalyst. In comparison to synthetic activators, biocatalysts have numerous benefits, for example, high particularity, high adjustability, and high catalytic activity for their usage in food industries, synthetic-chemical based industries, and pharmaceutics [103,104]. In any of their applications, properties like high reusability, better stability, and cost-

effectiveness are attracting researchers for manufacturing modern or advanced-level tools or technologies. The dynamic properties of enzymes are directly linked with biological applications. Furthermore, the dynamic behavior has a significant effect on the evolution of enzymes. The conformation alterations of enzymes majorly include allosteric regulation of the particular function of enzymes, catalytic movements, order-disorder transitions for effective chemistry, the multipoint reaction in the same enzyme, etc [105]. The active site of enzymes is generally composed of certain amino acids like serine (S), aspartic acid (D), glutamic acid (E), and histidine (H). These amino acids are responsible for the formation of ionic bonds, hydrogens bonds, and hydrophobic interactions with the specific substrate [106]. Enzymes are very special and follow a particular mechanism of action called interfacial activation [107]. The concept is defined as the improved activity of enzymes, particularly lipases onto insoluble/hydrophobic substrates like emulsions in comparison to the same types of substrates in the true monomeric form [108]. Owing to the interfacial activation, enzymes can manifest themselves in two different conformations; (i) open or active conformation- and (ii) closed or inactive conformation. The former conformation is more stable in comparison to the closed conformation [109]. The most crucial feature of major enzymes is the presence of a movable lid/flap which is located over the active site of the enzyme [110]. In homogenous media, if this lid (polypeptide chain) is closed, the active site is generally protected from the respective environment and is elusive to the specific substrates, hence the enzyme is inactive [107]. The changes in the conformation of the lid are due to the rigid movement of the helical part [111]. In the presence of a hydrophobic surface, the enzyme gets adsorbed on it, fixing a new structure (open firm) where the active center is fully exposed, thus being the enzymes able to hydrolyze drops of oils. On the other hand, when the lid is open, the enzyme is in the open conformation and the substrates can enter the active site of an enzyme and is further converted. This means the only open form can display the catalytic activity [112]. Generally, the open conformation of enzymes exhibits movement of the lid under the influence of hydrophobic surfaces/environment and thus increases the activity of enzymes due to interfacial activation [113]. Nevertheless, this particular movement does not alter the final characteristic properties of enzymes like specificity and selectivity. Such characteristic properties are readily modulated via genetic manipulation or certain physio-chemical modifications such as immobilization. Such a novel approach has already been exploited for immobilizing diverse enzymes on a variety of hydrophobic supports through the open conformation of an enzyme. Furthermore, the open form of an enzyme can stabilize the open form of other enzymes resulting in the creation of dimers (interfacially activating with each other nearby) [114,115] with altered catalytic features and active center. The resulting dimer is stable enough to utilize immobilized enzymes for selective adsorption of other molecules of enzymes. Another group investigated lipase-lipase interaction on glyoxyl-agarose support through multipoint interactions in the presence of Triton-X. The researchers observed the formation of bimolecular aggregates with improved adsorption capacity [116]. Another research group studied lipase-lipase interaction. They exploited lipase obtained from *Burkholderia cepacia* immobilized on glyoxyl-agarose support following incubation with diethyl-p-nitrophenyl phosphate (to block the active site) for adsorbing lipase enzymes. This adsorbed lipase has exhibited altered properties, high stability due to multipoint covalent attached, and interfacial activation [117]. Hence, this extreme alterations in the structure and properties of enzymes render a unique approach for modulating the enzyme characteristic features via controlled immobilization. Therefore, investigating free enzymes has become a complex task [107].

The innovation in the field of enzyme immobilization renders a viable technique to solve certain issues like operational stability, reusability, yield, activity, etc [118]. Hence, different microbial transporters and procedures are being utilized for enzyme immobilization to enhance the characteristics of the unbound enzyme [119,120]. The working of immobilized enzymes depends on certain characteristics like appropriate strategies of enzyme immobilization, pre-treatment before immobilization, transporter materials, and stacking on the microbial transporters. For example, earlier due to the periodate oxidation technique which had high activity retention but later employing carbohydrate moiety found the right integrated lipase [121]. For immobilization of *Candida rugosa* lipase than the permeable GMA-EGDM copolymer was replaced by the AGE-EGDM polymer particles because it has a high surface area [122] before the silica gel was covalently immobilized, the loss of lipase activity was restricted by the pretreatment of lipase with soybean oil on lipase enzyme. Moreover, movement recuperation and immovable proportion have been impacted by the transporter's superficial characteristics [123]. In this manner, misusing new carrier materials and immobilization strategies have significant importance on enzyme immobilization. This review gives an insight of a few significant points influencing enzyme immobilization and immobilized transporter materials, and numerous enzyme immobilization strategies [124].

Generally, it is of utmost requirement that the structure of the carrier must have enough mechanical strength, must be resistant to chemical attack, and decomposition from the attack of diverse microbes. Furthermore, various reactive groups and the hydrophilic chains are required in abundance on the surface of carriers for proper conjugation with the immobilized enzymes. A group of researchers have studied the effect of salts, solvents, pH, solubility, stability, and reusability of octyl-glyoxyl modified agarose beads by using calcium and magnesium chloride onto which they have immobilized lipase (*Rhizomucor meihei*). They have observed that both the salts have improved the stabilization (pH=5.0) of immobilized enzyme and are completely soluble though the preparation involving covalent attachment was much more stabilized (8-folds) in the presence of acetonitrile (ACN) [125]. Another group have produced structure lipids (SLs) for its applications in metabolic and inflammatory diseases owing to obesity. They have immobilized lipase (*Yarrowia lipolytica*) in calcium-alginate beads in the absence of any solvent. The immobilized biocatalysts have exhibited significant reusability (5 cycles) without any significant loss of its catalytic activity. Furthermore, the immobilized enzyme in calcium-alginate beads have exhibited fast acidolysis reaction, comparatively [126].

There are various techniques for enzyme immobilization onto a wide range of materials that have been created in the course of the most recent century. Enzyme immobilization includes the incorporation of the enzyme into different lattices or restricting its superficial region. A few adjustments like pre-manufacture of the matrix, integration of enzymes without expansion or inclusion of matrix had been manufactured is still persistently developing to refresh the conventions, so that immobilized enzymes become perfect for different rising uses. Enzyme immobilization is designated as an easy strategy and permits helpful treatment of enzymes. Two principal advantages are (i) simpler partition of enzymes from the item rendering their exploitation at large scale with diverse applications and (ii) limits the preparation of industrial item resulting in cost-effective (owing to the reusability of the enzymes), dependability (evaluated from the origin of the enzymes like animal, plant, or microbes) and productive procedure. In any case, barriers during mass exchange impacts are significant during the utilization immobilized enzymes. It can be blocked to a huge degree by the serious improvement of the immobilization procedure and consistent blending of the mixture having immobilized enzyme, substratum, and discharged item at the ideal speed [50].

There are various methods for the immobilization of enzymes, which are majorly divided into two groups: (i) physical methods-exhibits weak interactions between enzyme and matrix. It constitutes adsorption, entrapment/encapsulation [127]. In the adsorption process, the enzyme is adsorbed onto the surface of insoluble supports. It is a very simple method and operates with ease following broad applications. Enzyme immobilization is performed through simple mixing of enzymes with a specific adsorbent, under suitable reaction conditions such as pH, ionic strength, etc. After giving wash for removing loosely bound and free

enzymes, the immobilized enzyme is derived and is used in its native form. This process is based on van der Waal forces, ionic interactions, hydrogen (H) bonding, and hydrophobic interactions. Individually such interactions are weak but when combined all together they exhibit enough strength for binding between enzyme and matrix. Another method, entrapment or encapsulation is defined as the limited movability of enzymes in a porous gel still rendering them in a free form in the solution. The entrapped enzymes within gels are an acceptable method utilizing substrates and products with less molecular weight (MW). The difficulty of this process lies when large molecules approach the catalytic sites of an entrapped enzymes and hence inhibit the use of substrates with high molecular weight. Enzymes are generally entrapped in natural polymers such as agar, agarose, and gelatin via thermo reverse polymerization process, but in the case of alginate and carrageenan ionotropic gelation process is followed for entrapment [128]. There are diverse synthetic polymers that have been investigated for enzyme entrapment such as polyvinyl alcohol hydrogel [129], and polyacrylamide [130], and (ii) chemical methods-which forms a covalent bond between the matrix and the enzyme. It constitutes cross-linking and covalent bonding approaches. The process of covalent immobilization forms covalent bonds between the enzyme and the support matrix. Usually, the functional groups present in the enzymes are linked with the matrix since such functional groups have no potential in exhibiting the catalytic activity of an enzyme. The binding should take place in the reaction environment with no loss in the enzymatic activity. The reagents used in the process should have the least or negligible effect on the active site of an enzyme. The association of enzymes with the matrix via covalent linkage occurs due to side-chain amino acids such as arginine (R), aspartic acid (D), histidine (H), and degree of reactivity based on varied functional groups such as imidazole group, indolyl group, phenolic group, and hydroxyl group [131]. In this method, enzymes are attached through bi-functional or multi-functional reagents/ligands [128] and therefore, results in the formation of insoluble aggregates with high molecular weight. Another chemical method, cross-linking is usually not preferred since it does not use any support matrix for immobilization. They are generally gelatinous in nature instead of firm. As this process forms a type of covalent bond, the enzyme immobilized is regularly subjected to alterations in the conformation with loss of enzymatic activity [132]. Recently, a combination of these methods has been used in enzyme immobilization procedures. A team of researchers have immobilized lipase (*Rhizomucor meihei*) onto chitosan base supports in the presence of surfactants (Triton X-100 and sodium dodecyl sulfate) via two immobilization techniques. Firstly, the lipase was immobilized on the chitosan in the presence of surfactants through adsorption approach. Before immobilization, the base supports were functionalized with glutaraldehyde (cross-linker). Secondly, the immobilization of enzymes onto a glutaraldehyde-activated chitosan support (chemical cross-linking approach). The whole surface of enzyme is modified with cross-linker during the utilization of first immobilization approach, that is, adsorption. The addition of surfactant in low concentrations have resulted in increased biocatalytic activity in comparison to the free enzymes [133].

Depending upon the kind of interactions between carriers/support and enzymes, the enzyme immobilization techniques are further classified into two categories [134,135]; (i) reversible techniques-it is possible to separate enzyme from the base support/matrix under mild reaction conditions [136]. In this technique, the interaction takes place through weak non-covalent interactions such as hydrogen bonds, van der Waals forces, hydrophobic interactions [137], etc. Adsorption is a type of reversible immobilization technique [138,139]. and (ii) irreversible techniques- enzymes are adhered to base support/matrix and hence is very difficult to remove the enzyme without destroying either the matrix support or biological activity of the enzyme. Covalent binding, cross-linking [133,140], entrapment, and encapsulation are the type of irreversible immobilization techniques. The covalent bonds are extremely stable and ensure the strong binding of enzymes with the particular base support or matrix [141].

The characteristics of immobilized enzymes are determined by the sort of enzymes and lattice utilized for the immobilization. Advancement of any new immobilization conventions centers fundamentally around: enzyme recovery, working steadiness, acuteness, and decrease in restraint by items or some other part which a component of media (Table 1). It includes concentrated streamlining of immobilization mixtures for restrained cooperation among lattice and the enzymes and appropriate direction of the enzymes to keep up its synergist characteristics. In particular, utilization reagents that are unsteady and harmful during the immobilization procedures ought to be lying at a base, building eco-accommodating as for other elective advances [50].

Besides, reactors dependent on immobilized proteins have the least complex structure and the response is simpler to limit. Now and then, in the duration of arbitrary immobilization, unforeseen outcomes emerge, for example, a noteworthy decrease or an expansion in movement by a few folds, or abatement in enzyme dependability regarding solvent enzyme, and so forth. For instance, cross-connected crystals of subtilisin have 27 times less movement than that of the dissolvable enzyme; immobilized lipoprotein lipase utilizing a dissolvable interceded combination of esters has a superior action by 40 times as contrasted with protein substrate. The current part centers around ongoing advancements in immobilization strategies concerning the kind of matrices utilized (presentation of novel help like shrewd polymers) [50].

The first enzyme immobilization is being performed via one-point or a multipoint interaction, after that the support may continue to increase the number and quality of the particular interactions which involves new groups, for example, heterofunctional supports. Researchers, nowadays, have looked for varied types of solutions such as the novel design of heterofunctional supports [142–144]. It is defined as those types of support that exhibits distinctive functional groups on their surface which further have the potential to interact with the enzyme under diverse reaction environment. These kinds of supports are also termed multifunctional supports [145]. The ultimate aim of the researchers is to tackle certain issues such as leakage of an enzyme from the hydrophobic supports [146,147].

The first step of the process is to perform first immobilization through the immobilization following altering the reaction conditions.

Table 1
A summary of enzyme immobilization approaches discussing their merits, demerits, and numerous applications [4].

Enzyme immobilization method	Merits	Demerits	Applications	Ref
Solid surface deposition	Retaining almost all activity	Less enzyme loading	Carbohydrate's inversion	[5]
Physical entrapment	No negative impact on enzyme surface, thermally & mechanically stable	Restricted diffusion of the substrate to the enzyme	Capable for all enzymes & antibodies, development of biosensors	[10]
Encapsulation with lipid vesicles	The high degree of reproducibility	Enzyme inactivation through shear force	Medical, biomedical fields, enzyme-replacement therapy	[12]
Covalent immobilization	Stable to hydrolysis at neutral pH	Esters are not stable in aqueous conditions	Immobilization of antibodies, proteases, & oxidases	[8]
Immobilization through affinity tag	*In-situ* immobilization	Low selectivity	Capturing proteins during purification in affinity chromatography	[11]

Due to the presence of other functional groups, there is the possibility of the formation of new interactions with an enzyme resulting in the prevention of desorption of enzymes. Presently, all over the globe researchers are exploiting heterofunctional supports for immobilization of enzymes, for example, immobilization of lipase via covalent bonding on heterofunctional acyl-chemical reactivity which utilizes amino/glutaraldehyde moieties. These researchers have observed better performance such as better resistance towards detergent washing [148,149]. Another example immobilizes lipase B from *Candida antarctica* onto octyl-silica-amino-glutaraldehyde support displaying better stability [150,151]. Aghaei et al [152] prepared two synthetic layered double hydroxides, Mg/Al-CO_3 and Zn/Al-CO_3 through the co-precipitation method following their modification with sodium dodecyl sulfate (SDS) for the immobilization of lipase from *Candida rugosa* [153] through adsorption method. From the analysis, these modified base supports have shown high activity of an enzyme at a pH range of 6.0-7.0. Furthermore, these supports were resistant against the leaching of an enzyme [152]. Another group immobilized lipases A (CALA) and lipase B (CALB) from *Candida antarctica* onto magnetic nanoparticles functionalized with 3-aminopropyltriethoxysilane, activated via glutaraldehyde [153]. Other examples include lipase immobilization via covalent bonding on environment-friendly support (cashew apple bagasse) activated with glycidol-ethylenediamine-glutaraldehyde [154], immobilization of lipase (*Thermomyces lanuginosus*) onto divinyl sulfone (DVS) superparamagnetic nanoparticles which are further functionalized with polyethyleneimine (PEI) [155], adsorption of lipase (*Pseudomonas fluorescens*) on superparamagnetic $NiZnFe_2O_4$ octyl-nanoparticles through interfacial activation [156], immobilization of lectinase on Immobead-350 activated with epoxy, amino-DVS and amino-glutaraldehyde functional groups [157], lipase immobilization (*Rhizomucor miehei*) on magnetic nanoparticles coated with 3-aminopropyltriethoxysilane (APTES) [158], and immobilization of *Candida antarctica* Lipase B onto chitosan-divinyl sulfone via covalent attachment [144].

2.1. Methods of enzyme immobilization

Despite numerous points of interest in the utilization of enzymes contrasted with conventional catalysts, there are hardly any issues related to their utilization in modern uses. Enzymes are costly, which implies, the expense of their separation and decontamination is superior to standard synergists. The composition of enzymes is exceptionally delicate to different degrading conditions when disengaged from their common habitats. Their affectability to processing like pH, temperature, and substances escalates the cost of enzymes. Furthermore, most enzymes broke down in aqueous solution similar mixture of synergist frameworks, resulting in polluted products thus inhibiting the recovery of enzymes for their reusability, in the dynamic structure from the greater part of the reaction solutions [4].

One of the simplest techniques that appeared so far to beat such obstacles is the utilization of an immobilization procedure [159]. Immobilization is a process where enzymes are fixed to or inside strong support/matrix, making a diverse immobilized enzymatic arrangement. The common mode in living cells imitates the type of immobilized enzymes, hence the greater part of these are appended to organelle structures, cell cytoskeleton, and membranes. The strong supportive frameworks for the foremost part balance out the structure of the enzymes and, as an outcome, continue their exercises in this manner when compared with the free enzyme. In the liquid mixture, immobilized enzymes are progressively strong and are immune to ecological effects. Also, mixed immobilized enzymes frameworks permit the straightforward reusability of enzymes and hence can be used for various cycles with persistent activity of catalytic procedures, fast end of responses, and more noteworthy assortment of fermenter plans but in comparison to the free catalysts, immobilized enzymes show decreased action with higher evident Michaelis constants on account of relative trouble in going to the substrate [160].

The conjugation of enzymes onto various matrices through physical interactions including hydrogen (H) bonding, hydrophobic interactions, and van der Waals forces. The nature of the procedure is reversible by administering biochemical variables [161] (Fig. 1). The characteristic features of immobilization technique constitute simple procedure, no change in the internal properties of enzymes, no chemical alteration, and lattices are easily accessible in several structures. The various hindrances of this strategy are; enzyme spillage, fragile balance among mechanical characteristics of the pattern, and its impact on enzymatic action [162]. The technique adsorption is basic with an incredible assortment of microbial transporters accommodating synchronized refinement even as immobilized enzymes (for example, CM-cellulose having Asperginase) with no change in configuration. Nonetheless, it includes escalated streamlining due to the association of diverse variables that assumes employment in elution of enzymes resulting in minor transitions in its microbial habitat such as high substrate fixations, ionic quality, pH, and temperature [163].

It creates a bond of enzymes onto various grids involving ionic or covalent bonds and therefore the method is irreparable [161]. The covalent technique for immobilization prompts more noteworthy enzymatic strain and results in extreme changes in conformational and catalyst characteristics of the protein, due to strict immobilization situation and simultaneousness of comparable class of amino at the dynamic point which included at the time of interactivity of enzymes with the lattices [164]. The main benefit of the interconnecting technique is its simplicity because it prompts the passing of a lot of enzymes due to irregular reactions. Hence, this system for enzyme immobilization endures barriers caused by dissemination [4,165,166].

Presently, enzyme immobilization consists of two types of strategies namely (i) carrier-bound immobilization and (ii) carrier-free immobilization [74]. The carrier-bound immobilization is usually done by entrapping the enzyme into polymeric matrices. Though the immobilized enzymes onto non-catalytic matrices have certain benefits, the volumetric activity of the biocatalyst and the productivity of a particular reaction can be decreased because of the presence of the non-catalytic mass of the carrier [68,85,167]. Hence, the carrier-free enzyme immobilization approach has grabbed the interest of numerous researchers. Usually, the carrier-free enzyme immobilization does not require additional inactive mass as a carrier rather they are assembled by direct cross-linking with various preparations of enzymes. Currently, the carrier-free enzyme immobilization constitutes (a) cross-linked enzyme (CLE), (b) cross-linked enzyme crystals (CLECs), (c) cross-linked spray-dried enzyme (CSDE), and (d) cross-linked enzyme aggregates (CLEAs), respectively [42,168].

Cross-linked enzyme aggregates (CLEAs), was first demonstrated in the 2000s, and have grabbed the attention of researchers all over the globe [169,170]. The preparation of cross-linked enzyme aggregates consists of precipitation/aggregation of an enzyme following chemical cross-linking of the resulting aggregates of enzymes [171]. The aggregation is induced through the addition of a precipitant like salts, organic solvents, or non-ionic polymers to an aqueous solution of an enzyme. The resulting enzyme aggregates are consequently cross-linked with a bi-functional agent [172,173]. The CLEA has advantages such as simplicity, robustness, stability towards heat denaturation, operational stability, volumetric productivities, and recoverability, reduced cost of production because of the absence of a carrier, more activity of concentrated enzymes, and proteolysis [174–178]. The significant reason for better stability of CLEAs is due to (a) rigidity of the tertiary structure of enzyme owing to the extensive cross-linking [179], (b) prevents the dissociation of multimeric enzymes [180], and (c) prevents the denaturation of enzymes via multiple attachments of the molecules of enzymes [181]. Nevertheless, the CLEAs has certain drawbacks like frail technology for numerous industrial applications [182], difficulty in handling and recovering the particle completely from the reaction media [183–186] due to small particle size (<10 μm) [187], restricted internal mass-transfer of CLEA and thus reduced catalytic efficiency

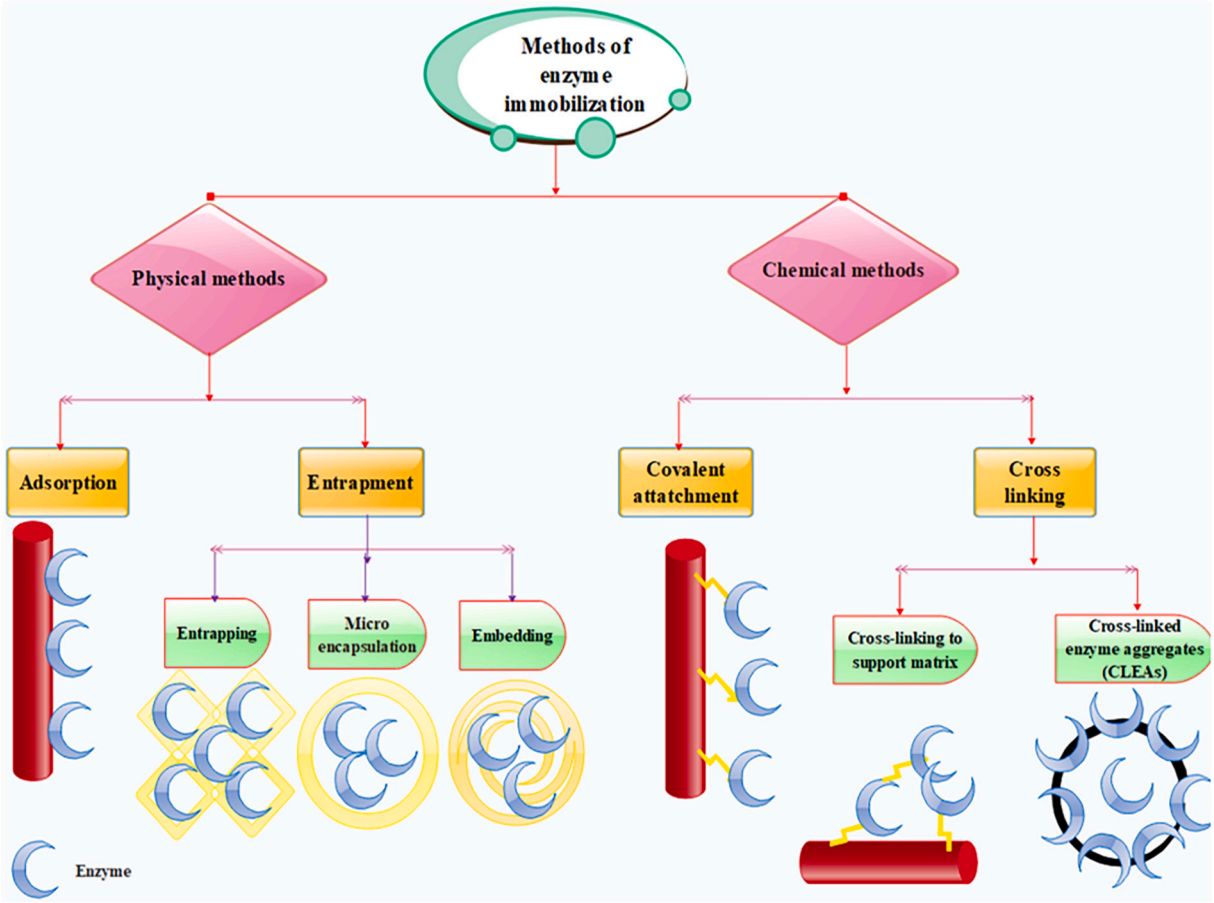

Fig. 1. Different methods of enzyme immobilization [4].

[174]. Thus, there is a dire need of improving the CLEA.

In the recent past, CLEA has exploited with a diverse array of enzymes like hydrolases, oxidoreductases, lyases (*Rhodotorula glutinis*) [42], penicillin acylase [188], lipases (*Candida antarctica* lipase B) [189–191], lipases from *Pseudomonas* [192,193], lipase from *Rhizomucor miehei* [194,195], *lipase from Chromobacterium violaceum* and *Chromobacterium amazonense* [196], laccases [197,198], alcohol dehydrogenase [199], and nitrilase [200], phospholipases [201,202], esterases [203]. Nevertheless, few disadvantages restrict their use in industrial applications, for example, reduced efficiency of cross-linking resulting in significant loss of enzyme activity, poor compressive resistance which results in reduced catalytic efficacy, difficulty in modifying important ε-amino groups via glutaraldehyde which ultimately results in loss of biological activity [204]. Intending to overcome the aforementioned drawbacks, few amendments have been done by the researchers for improving the cross-linked enzyme aggregates (CLEAs) technology such as co-aggregation of enzymes and polyethyleneimine or bovine serum albumin (BSA) which will improve the cross-linking efficacy [205], sugar is added which acts as a stabilizer and hence will minimize the enzyme activity loss [206]. However, such methods are not enough to enhance the enzyme activity and stability [207,208].

2.2. Enzymatic support materials

Immobilized transporter materials are formed by the contribution of immobilized enzymes (Fig. 2). The advantage is that there are easily accessible, non-toxic, biocompatible with the particular enzyme, and biodegradable [209] (Fig. 3).

Various supports have been reported for enzyme immobilization such as (i) natural polymers which constitute cellulose, sepharose,

Fig. 2. Some fundamental features of enzymatic support materials [210].

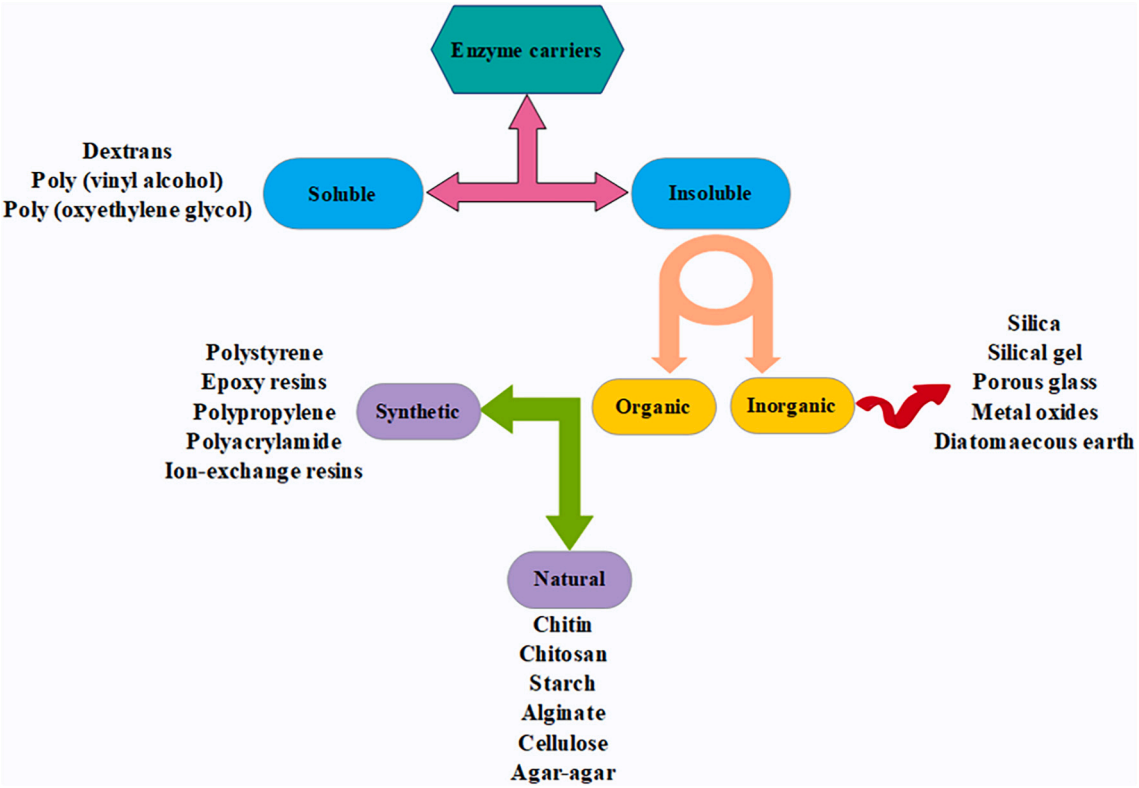

Fig. 3. Enzymatic microbial transporters for enzyme immobilization [124].

sephadex, pectin, alginate, starch, carrageenan, chitosan [144,211], *Hypericum perforatum* [212], Triton-X-100 [213] and gelatin, (ii) synthetic polymers including amberlite, DEAE cellulose, polyethylene glycol (PEG), and polyvinyl chloride (PVC), methacrylate [214], nanofibers [215], nanotubes [216], octyl-modified mesocellular foams [217] (iii) polyacrylamide gels [218], (iv) inorganic materials like zeolites, ceramics, celite, silica, glass, activated carbon, and charcoal [219] and (iv) nanomaterials like gold (Au) nanoparticles [220], carbon dots, silver (Ag) nanoparticles immobilization of enzymes like lysozyme [221], glucose oxidase [222], aminopeptidase [223], and alcohol dehydrogenase [224], cadmium sulfide (CdS) nanodots, carbon tubes, magnetic nanoparticles [158,225–227], nanofibers, Janus nanoparticles [228]. Gold nanoparticles have been used widely as base support owing to their biocompatibility, surface area, and quantum size effects. A classical research work deals with the immobilization of laccase with gold nanoparticles exhibiting nearly a 2-fold improvement in the catalytic activity [229]. Kang et al. linked laccase with the -PO_3 group present on the surface of the carbon dots and ensures improved transfer of electrons and affinity towards substrate [230,231]. Titanium oxide (TiO_2) nanoparticles have been exploited for immobilizing certain enzymes namely peroxidase, trypsin, cellulase, lipase [232], and α-amylase. These immobilized enzymes have displayed better enzymatic activity in comparison to the free enzymes. Additionally, these immobilized enzymes have improved thermal stability [233].

As a bit of the immobilized enzyme, the shape and character of the transporter impact the enzymatic properties. A couple of characteristic inorganic molecules and polymer materials are usually utilized as sustaining substances [234]. Additionally, numerous scientists had developed enormous concern in utilizing different integrated materials of polymer which will work like transporter for great mechanical stability and efficient movable characteristics [235]. Particularly, inorganic-natural composite materials have pulled in profound consideration [236,237].

Considering polymeric materials, extensive efforts are being made to exploit starch, chitin, chitosan, and cellulose due to non-toxicity, fewer impurities, easy alterations, abundant sources, presence of functional groups ensuring modifications, etc [238]. Synthetic polymers are found out by catalytic processes utilizing different monosaccharides exhibiting excellent physical unbending, superior explicit region, easy to amend the surface properties, and its capabilities for carrying functional groups as demanded by needs. Henceforth, it has been broadly explored and utilized for enzyme immobilization [124].

2.3. Characteristics of enzyme immobilization

During the immobilization of enzymes, there is an adjustment in the microbial habitat of enzymes due to the fluctuated cooperation of the networks with the substrates hooked into their chemical and physical characteristics. This section discusses significant conditions which are influenced during the enzyme immobilization process [50].

2.3.1. Optimal pH

pKa of amino acids affect the pH within the region of the enzyme's dynamic surface. The adjustment is ideal pH has been seen on account of immobilized proteins (it is which expanded or moves to basic or acidic side) regarding dissolvable enzymes. The degree of progress relies upon the type of immobilization technique utilized even as the chemical and physical characteristics of the matrix [135]. The adjustment is ideal pH profile is characterized by the equation:

$$\Delta pH = pHi - pH0 = 0.43\frac{\varepsilon\varphi}{kT}$$

kT, when charged matrices (either negatively or positively charged), pH_i is that the pH in the region of proteins which are immobilized, the mass in a liquid mixture of pH is pH_o, a positively charged proton, is ε, electrostatic potential stands for ϕ, whereas, the temperature is T and Boltzmann constant is k [50].

Additionally, when the matrix is charged the ideal pH moves to the acidic site. The degree of moving relies upon the thickness of the

charged region of the matrix. Widening of the perfect pH is seen as and when extremely better stacking of protein, i.e., a huge amount of protein action per part of the lattice. It prompts better natural explicit movement (Zulu effect), it builds the convergence of discharged molecules of product close to the protein of immobilization. The development of substratum atoms to the dynamic region of the protein which is immobilized is deterred due to the better convergence of protein (present inside the matrix or on a superficial level) and molecules of the outcome. Along these lines, the pace of the response is seen to be consistent for a more drawn-out span notwithstanding the adjustment within the pH of the answer [239,240].

2.3.2. Optimal temperature

The movement of immobilized enzymes in the ideal temperature to upper temperatures. While immobilization of protein, unbound development of molecules of protein is hindered, is also visible at upper temperatures. During this manner, denaturation of protein isn't seen due to the security of amino acids at the dynamic superficial region, while substance transition occurs at upper temperatures so as when the temperature builds molecules of substance increases the kinetic energy and reach the dynamic region of the immobilized proteins quickly, that moves the perfect temp to upper temps. The degree of ideal temp uprooting the immobilized proteins relies upon the type of lattice even as on the interactivity of the lattice and the protein. Expanding of the perfect temp. extend has been additionally seen in different scenarios due to the better natural explicit movement (Zulu effect) with the conversation like ideal pH [239].

2.3.3. Kinetic parameters

Changes in kinetic parameters (K_m, V_{max}, k_{cat}) are seen during the immobilization of protein. Immobilization of protein doesn't guarantee that molecules of enzymes are appended in their right compliance (that is including buildups from the dynamic site), which firmly influences the V_{max} of the enzyme. Moreover, dispersion boundaries (inner and outer) are other significant purposes behind changes in kinetic parameters [241,242]. If there should be an event of an inner dissemination hindrance, the protein is available within the matrix, which restricts the dispersion of molecules of the substrate into the lattice, which influences K_m led by V_{max} [243]. In any case, increment in mixing would help in decreasing the impacts caused by outer dissemination due to the subsequent connection. Here, the diffusion constant is alpha (α):

$$\alpha = \frac{\mathrm{Vmax}}{Km}$$

Besides, lattices of polyionic additionally influence dynamic conditions of proteins that are immobilized due to dividing impacts, which incorporates communication between the matrix and therefore the ionic substratum even as result molecules [243]. In the nearness of dividing impacts, the Michaelis-Menten equation becomes:

$$\mathrm{v} = \frac{(\mathrm{Vmax})\ \mathrm{X}\ (\mathrm{So})\ \mathrm{X}\ (\mathrm{p})}{Km + So\ X\ p}$$

$$Km\ (app.) = \frac{\mathrm{Km}}{p}$$

Here, the partition coefficient is p (p = Si/So, where Si is that the concentration of ions around enzyme so the thickness of ions in large quantity stage). Hence, excessiveness in separating follows to lessen the K_m and ascend the V_{max}.

2.3.4. Stability parameters

The steadiness of the immobilized enzymes is dependent on the matrix type, and the matrix between the lattice and enzymes which requires receptive, steady, and insignificant obstructions in the arrangement of atoms during the reaction. When the enzyme is properly immobilized, the matrix is inactivated and hence no obstruction in the arrangement whereas biocatalysis exploits functional groups which are available on the lattices via utilizing reasonable chemical reagents [95,241].

The conditions of enzyme immobilization constitute (i) response time- should be ideal, and ensures precise arrangement of the superficial region of the lattice, partially solidified enzyme and enzyme which is already immobilized, (ii) pH-keeps on changing with every enzyme immobilization framework because it changes due to enzymes used, enzyme carrier, and type of enzyme immobilization approach, (iii) thermal capacity- needs to be streamlined for various immobilization frameworks. With stabilized temperature, there is a better chance of acquiring better enzyme bearing bonds due to better oscillation pace in-between molecules of enzymes even as the lattice, (iv) buffers: are important for enhanced stability and efficiency of enzyme immobilization such as meddling of borate between the catalysis of NH_2 and -CHO yet with the help of tris ethanolamine [244], (v) storage stability- it is difficult to reuse free enzymes effectively for numerous industrial applications [84] whereas enzyme immobilization helps in tackling this disadvantage by enhancing the reactivity and stability of an enzyme [49,245]. The immobilization approach prevents the enzyme from denaturation of the structure due to the external environment and hence maintaining the activity of enzymes from diverse reaction conditions by sustaining the storage stability [246,247]. There is diverse research work that has observed the improvement in storage stability such as the use of functionalized iron oxide nanoparticles modified with carboxymethylated chitosan as a carrier for conjugating papain via covalent interactions [248]. In comparison to the free papain, the modified nanoparticles which are immobilized with papain have shown improved activity of enzymes and storage stability[124,249], and (vi) denaturants-immobilized enzymes are more stable than free enzymes. Concerning the stable conformation enzymes, the immobilization reactions are usually carried out under mild reaction conditions. Numerous researches have been performed, for example, entrapped peroxidase enzymes in calcium alginate-starch hybrid supports are comparatively more stable under the influence of denaturants such as urea owing to the internal carbohydrate moieties [219,250].

3. Polysaccharides and their types

The characteristic properties of the matrix have cardinal significance intending to determine the performance of the enzyme immobilization system. The ideal characteristic features of base supports are physical resistance to compression, hydrophilicity, inert environment in the near vicinity of enzymes, easy derivatization, biocompatibility, resistance against microbial attack, easy availability, and less cost. Although, enzyme immobilization onto solid base supports is an established and emerging technology, there are still to date no general rules to select the best base support for a particular application [251–253].

Selecting a suitable method for the purification of biomolecules is a crucial step intending to evaluate the economic behavior of both upstream and downstream processes, respectively. The biosynthesis of the favorable product during the production of enzymes majorly constitutes diverse stages of separation like downstream processing which is expensive, laborious, and time-consuming. Nevertheless, the shelf-life of such methods is typically dependent on the choice of purification technique and reaction condition of a production process [254]. There is a dire need to purify enzymes for their robust exploitation in the commercial market. Though there are numerous traditional and advanced-level methods to solve certain issues or challenges (enzyme loss, linkage of enzymes to the appropriate matrix, etc.), still the purification step of enzymes remains a significant obstacle. The enzyme purification generally helps in minimizing the loss of the enzyme during the entire process [254,255].

There are various methods to purify enzymes but the commonly exploited are ammonium sulfate precipitation, ultrafiltration, and ion-exchange chromatography [256]. In the case of ammonium sulfate

precipitation, there are two particular applications: (i) total precipitation where enzymes are precipitated through the addition of the salts, and (ii) the precipitated enzymes are further recovered through centrifugation following resuspension in a minimum volume of water and hence are concentrated. The leftover salt is removed via dialysis by interfering with the activity of the enzyme and its consequent steps of purification [257,258]. The added salts result in the precipitation of enzymes via removal of water from the hydrophobic portion on the surface of proteins which results in insolubility [259].

The principle of ultrafiltration relies on the separation mechanism via a semi-permeable membrane having a specific molecular weight (MW) cut-off point, hence, separating the extract based on particle size instead of charge [260] (Table 2). Ion-exchange chromatography- a non-affinity absorption strategy that purifies enzymes based on molecular charge instead of molecular weight (MW). The resin in the column is either negatively charged or positively charged. Proteins also exhibit some charge depending on the pH of the solution. The major principle is to pass the extract of protein from the column at a particular pH where they will show maximum adherence to the oppositely charged matrix. The pH is altered gradually during separation and the proteins are eluted via charge neutralization by changing the pH of the elution buffer. All necessary fractions are collected from the chromatographic column and further scrutinized for enzyme activity for identifying the fraction of interest [260,261]. At the same time, diverse novel technologies like ultrasound-assisted extraction, hot-water extraction (little time consuming and not applicable to all types of polysaccharides), ethanol precipitation, deproteinization by the Sevag method, trichloroacetic acid method, hydrochloric acid (HCl), and pulsed electric energy have also been exploited for purifying polysaccharides [262,263].

Generally, polysaccharides are hydrophilic macromolecules. The approaches to purify polysaccharides vary in comparison to small molecules. Additionally, different polysaccharides have different characteristic properties and hence require a different method for their purification. The immobilization of enzymes is quite difficult as it has very few lysine (K) residues. The functional groups in the structure of lysine have a significant role in the bonding of enzymes with the matrix.

Table 2
Different types of purification technologies and their respective advantages and disadvantages [264].

Purification methods	Advantages	Disadvantages
Graded precipitation	Easy operational process	Difficult collection of homogeneous fractions
Salting-out precipitation	Cost-effective	Low efficacy, easy co-precipitation
Metal coordination	Better specificity	Difficult to find a suitable coordination reagent.
Quaternary ammonium salt precipitation	Purification of acidic and neutral high molecular weight polysaccharides. It has better selectivity	Difficulty in controlling the ionic strength and pH of the solution.
Anion exchange column chromatography	Majorly used, ideal for purifying diverse acidic or neutral polysaccharides	Height of column bed changes with altering pH/ buffer
Affinity column chromatography	Easy operational process, high efficacy	Issues of finding an appropriate ligand for specific polysaccharide
Ultrafiltration	Based on molecular sieving	Poor yield, laborious process
Ultracentrifugation	Improved efficacy	Requirement of bulky equipment's, majorly exploited for semi-micro preparation of polysaccharide
Preparative zone electrophoresis	Better separation effect	A time-consuming process, minimum purification capacity, majorly exploited for semi-micro preparation of polysaccharide

Polysaccharides, nowadays, are utilized as base support materials for producing recoverable biocatalytic systems with improved stability of an enzyme under suitable reaction conditions. The significant advantage of exploiting polysaccharides is (i) availability in abundance, (ii) easy availability, (iii) biocompatible, (iv) no requirement of separate purification or any advanced-level treatments rendering them a cheap option as base-support materials [265,266], and (v) presence of various types of functional groups, for example, hydroxyl group (–OH), carboxylic acid group (COOH), carbonyl group (C=O), thiol group (–SH), and amine group ($–NH_2$) on the surface of the polysaccharides acknowledges the formation of covalent bonds between enzyme and the base support and facilitates the modification of polysaccharides. Due to the introduction of functional groups, there has been a significant reduction in the steric hindrances due to increasing area for adhesion and hydrophobicity of the support [210,267]. Therefore, polysaccharides are recognized as a desirable base support for particular applications of enzyme immobilization such as anti-obesity [268], food and pharmaceutical industries, environmental monitoring via molecular docking studies [269], anti-inflammatory, anti-bacterial, anti-viral, protection against heart and liver disorders [270], managing environmental pollution by fabricating biodegradable plastics in the presence of *Fusarium solani* cutinase and *Candida antarctica* lipase [271], etc.

The standard biopolymers holding the dominant part of nourishments are polysaccharides with an exception of meat and dairy-based nourishments. Polysaccharide solutions are normally compositionally, spatially, and progressively intricate. The composition and structure of polysaccharides are both polymolecular and polydisperse with spatial intricate network thus ensuring significant interactions with water and different molecules in a particular environment that further impact their shapes with varying degrees of mobility, temperature, etc. The movements of polysaccharide chain portions in semi-solid food frameworks cover a good scope of frequencies and are significant supporters of the mechanical reactions and thermodynamics of the framework [272].

Additionally, the enzyme immobilization approach is reproducible but not sophisticated equipment hence, required a simple method for enzyme immobilization. Therefore, numerous supports can be adopted for carrying out the enzyme immobilization process, for example, immobilization of lipases (*Candida antarctica* (isoform B)) and *Thermomyces lanuginosus* (TLL) [14] onto octyl support via covalent attachment and interfacial activation [273], covalent immobilization of porcine pancreatic lipase onto carboxylated silica-coated magnetic nanoparticles via 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride/*N*-hydroxy succinimide (EDC/NHS) coupling reaction [274], and interfacial activation and adsorption of lipase with triglyceride-water interfaces [275]. These are comprehensively arranged as (Fig. 4) (a) glycans of plant source: exudate gums like arabinogalactan, gum acacia (GA), gum arabic, and locust bean gum (Fig. 5) cellulose and starch, (b) glycans of algal source: galactans—carrageenan, alginates (c) glycans of animal source: hyaluronic acid (HA), chitosan, glycosaminoglycans and chitin, (d) glycan of microbe source: pullulan, dextran, gellan gum and thickener [276].

The most common and abundant form of glycans found in plants is starch. While connecting by α-(1,4)- glycosidic linkage and partially by α-(1,6)- glycosidic linkage we can find starch by rehashing α-d-glucose units. Starch comprises two particular glycans: amylopectin and amylose (Singh, 2011). An exceptionally branched polymer having a relative molecular mass varying from 107–109 g/mol is amylopectin [277,278]. Immobilization of α-amylase starch has been explored, due to which temperature inactivation leads to higher resistance because of covalent immobilization [279]. Mixing with calcium alginate beads and crude potato starch leads to immobilization of α-amylase with the help of *Bacillus amyloliquefaciens*. It created the impression that the protein which is immobilized indicated a more active nature in comparison to the free enzymes, and the 40% productivity after the seven cycles is held due to alginate beads [280].

Organic glycans are chitosan and chitin. One of the most bounteous

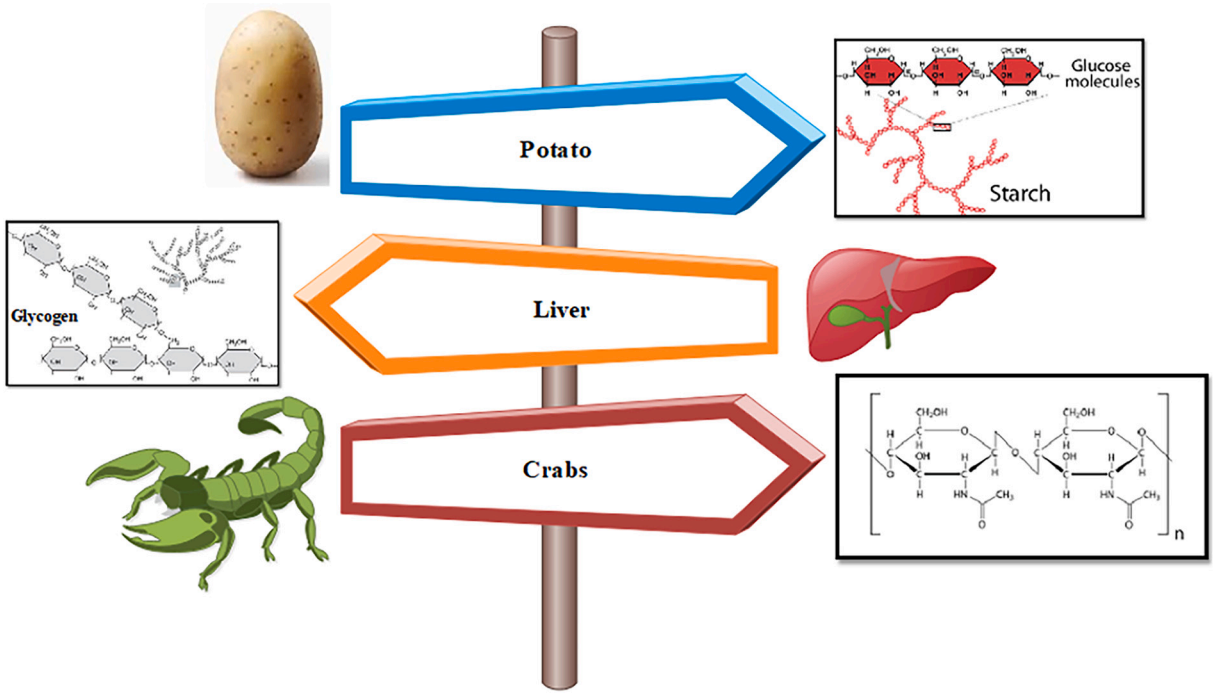

Fig. 4. Diagrammatic representation of a broad classification of polysaccharides [276].

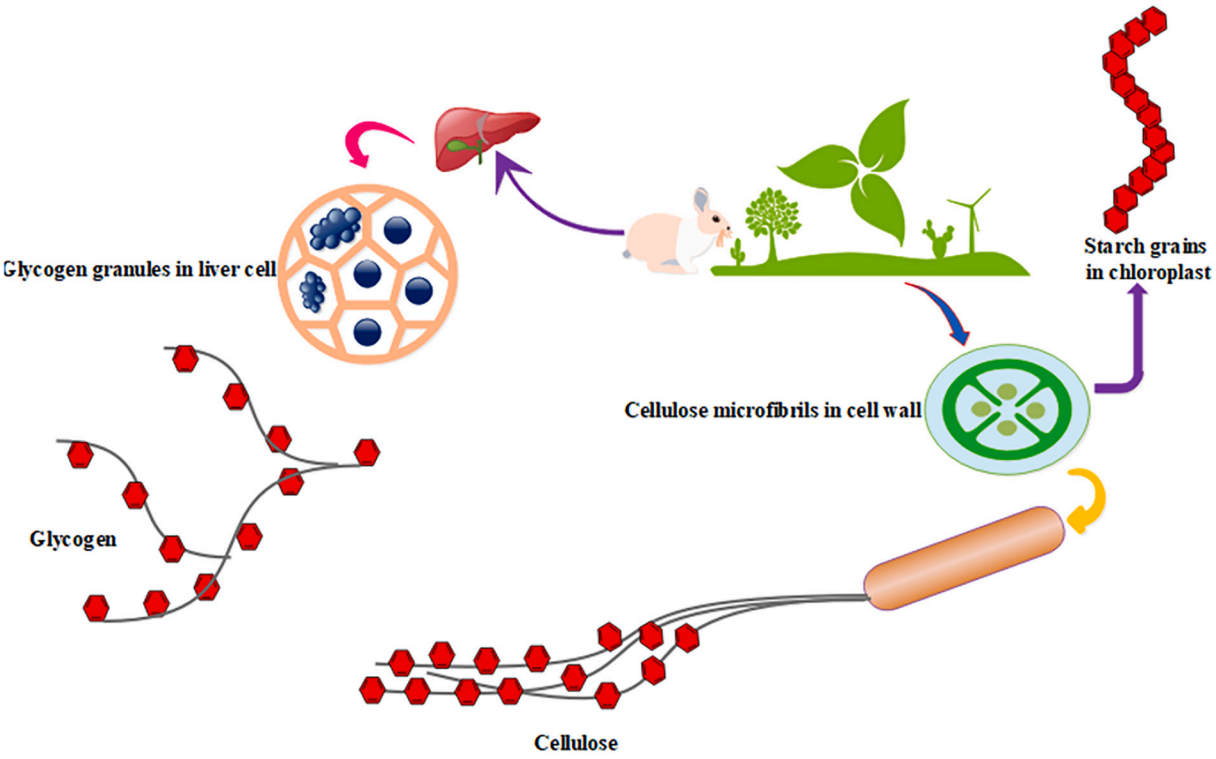

Fig. 5. Diagrammatic representation of polysaccharides of plant origin [276].

renewable natural resources is chitin, whereas its main subsidiary of chitin created by N-deacetylation to a fluctuating degree is chitosan. Safe, hydrophilicity, physiological inactivity, gel-shaping characteristics, biodegradability to benign, and uncommon fondness to enzymes are the many attributes of chitosan and chitin. Because of these attributes, chitosan-and chitin- made substances are broadly used as supports for protein immobilization. Profoundly expanded beads of chitosan are arranged from the source of cuttlefish squanders interlinking with glutaraldehyde or glyoxal showed an honest arrangement of fondness toward β-glucosidase and acid phosphatase [281].

The most abundantly found biopolymer is cellulose which is widely used for protein immobilization which is a result of its mechanical properties, safe for the environment, non-toxic, less-temperature development, and less heavy in weight. Different alterations had gotten

alluring properties of cellulose for enzyme immobilization. diethyl aminoethyl (DEAE)- altered cellulosic supports immobilization of fungal laccases [282]. Electrospun cellulose membranes are products of regenerated cellulose that have better action in the retention of lipase [283]. Working with gold nanoparticles with cellulose nanocrystals (CNC's) helps out cyclodextrin glycosyltransferase (CGTase) by filling in, and liquor oxidase with no observable loss of the original action of the enzyme. On joining of polyacrylic acid adsorbed lipase with very thin cellulose fibers are joined showing exceptionally improved dissolvable steadiness over unrefined lipase [284]. Carbon nanofibers arranged to employ physical and oxidation crushing of cellulose have demonstrated broad protein immobilization action and to α-chymotrypsin's stable nature [285]. The immobilization of lipase hydrogel beads in bacterial cellulose chitosan by chemical and physical adsorption and covalent bonding is used for several organic, bio-catalytic, and biomedical applications [286].

The significant breakthroughs in the field of enzyme immobilization have resulted in increased recovery and reusability of enzymes following a reduction in the cost associated with the use of enzymes which ultimately fuels the growth of biocatalysis. Nevertheless, the present-day immobilization approaches (also termed random immobilization) suffer from leaching (due to the formation of planar aggregates through multiple sites which requires simultaneous desorption for the release of the aggregates), stability, recoverability, and reusability problems [83]. Furthermore, such techniques do not have sufficient control over the orientation of the immobilized enzymes. At present, to address such obstacles, researchers are putting extensive efforts on site-directed immobilization of enzymes via particular techniques. This particular approach ensures proper control over the orientation of immobilized enzymes which will further strengthen the enzymatic activity, stability, and selectivity of enzymes by minimizing the impact of the steric hindrance of the active site of enzymes after the immobilization process and hence renders a better understanding of the interactions between enzyme and the appropriate surface or carrier such as polysaccharides (chitin, chitosan, gelatin, pectin, sepharose, etc) [287]. The major advantages of polysaccharides are less toxicity, abundance, sustainability, less chemical reactivity, biocompatibility, biodegradability, and a simple process of enzyme fixation. Additionally, the presence of free amino ($-NH_2$), hydroxyl (-OH), the carboxylic acid (-COOH) functional groups on the surface of polysaccharides renders a versatile platform for binding of enzymes properly during the site-directed immobilization process ensuring the stability, selectivity, activity, and proper orientation of enzymes [288].

Owing to the escalating demands of novel materials for their applications in diverse fields such as food industries, pharmaceutics, textile industries, healthcare [289], etc extensive efforts are being made by global researchers for manufacturing advanced-tailor-made functional material with extraordinary properties [290]. Here, advancement in the development of supports/carriers derived from polysaccharides is being done due to numerous advantages of polysaccharides and hence are majorly exploited in developing robust catalysts, diagnostic devices, biosensors, light-weight cost-effective medical devices, etc [291]. The presence of various functional groups on the surface of polysaccharides ensures the linking of enzymes via an appropriate method such as covalent linkage, cross-linking, adsorption, etc. These functional groups behave as a linker molecule between polysaccharide support and specific enzymes for robust immobilization [292]. For example, carboxymethyl cellulose (CMC) ensures immobilization of particular bioactive molecules like antibodies on the surface of cellulose via adsorption for diverse immunodiagnostic applications [293]. Furthermore, to sort the issue of random orientation, another study was conducted where the bioactive molecules were immobilized through the utilization of two approaches; (i) adsorption via carboxymethyl cellulose, and (ii) avidin-biotin interaction. The researchers have studied its applications for developing biosensors for the detection of hIgG (human immunoglobulin-G) from fluid matrices [294]. A classical documented work has discussed the immobilization of laccase enzyme via entrapment into chitosan/nitrogen-doped carbon hollow spheres for electrochemical detection of craft lignin (biosensor). In another study, laccase was entrapped in copper-alginate beads via physical entrapment for its applications in the degradation of dyes like Indigo Carmine [295].

4. Utilization of polysaccharides in enzyme immobilization

4.1. Polydopamine coated with magnetic-chitin (MCT) particles for enzyme immobilization

Carbohydrate biopolymers, for instance, starch, chitin (CT), and cellulose are exhibited as indispensable common substances within fields, chemistry, biotechnology, and biology. Polymers like chitin are one of the most inexhaustible biopolymers on the planet. Chitin is a safe and environment-friendly muco-glycan, i.e., the arthropods segment, bug skeletons, and fungal cell walls. chitin is formed of α (1→4) linkage 2-acetamido-2-deoxy-D-glucose units. Chitosan and chitin are oftentimes contrasted with cellulose and structurally vary just at C2 carbon place. Even though CT has been delivered at least 1010 tons yearly [296], low consideration was given to its uses and was presented as an unused natural asset. Due to solid intermolecular and intermolecular hydrogen bonding gives an inflexible polymer structure, which provides unavailable shape arrangement for a few reagents and solvents. The N-acetyl group exists at the C2 carbon position of chitin limits the immediate use of the polymer. Deacetylation of the acetamido groups of chitin delivers the financially significant chitosan. Chitosan was researched as broadly as cellulose for modern and laboratory uses [297].

The marine adhesive protein which contains the catechol and amine functional groups has been demonstrated to be a fruitful glue compound that can be joined onto different surfaces and has been analyzed as the main ingredient of dopamine [122]. At a typical marine pH condition, to make the reactive quinones and free radicals, basic oxidative self-polymerization of dopamine (pH 8.5, 2 mg/ml 10mM Tris) was considered. Electron-lacking ring and electron-giving amine groups are held by dopamine quinone. To frame dopaminochrome requires the cyclization reaction followed by oxidation of deprotonated amine groups. The said item will promptly change and create a dark and thin adherent polydopamine membrane at this point over five,6-dihydroxyindole. Accurate chemical, dopamine, and physical polymerizations are not surely known. In any case, to various active surface functional groups, the polydopamine layer was accepted, for example, catechol, amino, and imino for secondary reactions; these might be advantageous [298].

By exploiting the actual proclivity interactivity among lysozyme and chitin, out of this chitin broke down in liquid iron mixture was supplied to magnetic chitin (MCT) after co-precipitation to MCT's molecules is effectively recoverable partially, surface assimilative for the disconnection of lysozyme from the albumen. N-acetylated subunits of chitin comprise the larger parts of amine groups, chitin will not provide another reactive functional group that can be utilized as immobilized biomolecules (Fig. 6). For biomolecule immobilization which can broaden the biotechnological utilization of chitin, MCT is a viable and helpful carrier that can be obtained from clinging polydopamine i.e., made on the surface of MCT [299].

A legendary strategy for the superficial adjustment of CT has been created. For immobilization of α-amylase clinging polydopamine magnetic chitin particles that are recoverable and surface functionalized have been used effectively. By pre-treatment of the polydopamine surface with glutaraldehyde; the immobilization effectiveness can be improved additionally. By moving 0.5 the pH unit to the acidic area, the ideal pH of the immobilized enzyme can be obtained. With the increase in pH and temperature the immobilized enzyme more effectively hydrolyzes the starch. Various characteristics like magnetic reusability and unrivaled sturdiness can be seen on polydopamine functionalized MCT microparticles when the immobilized amylase is treated on

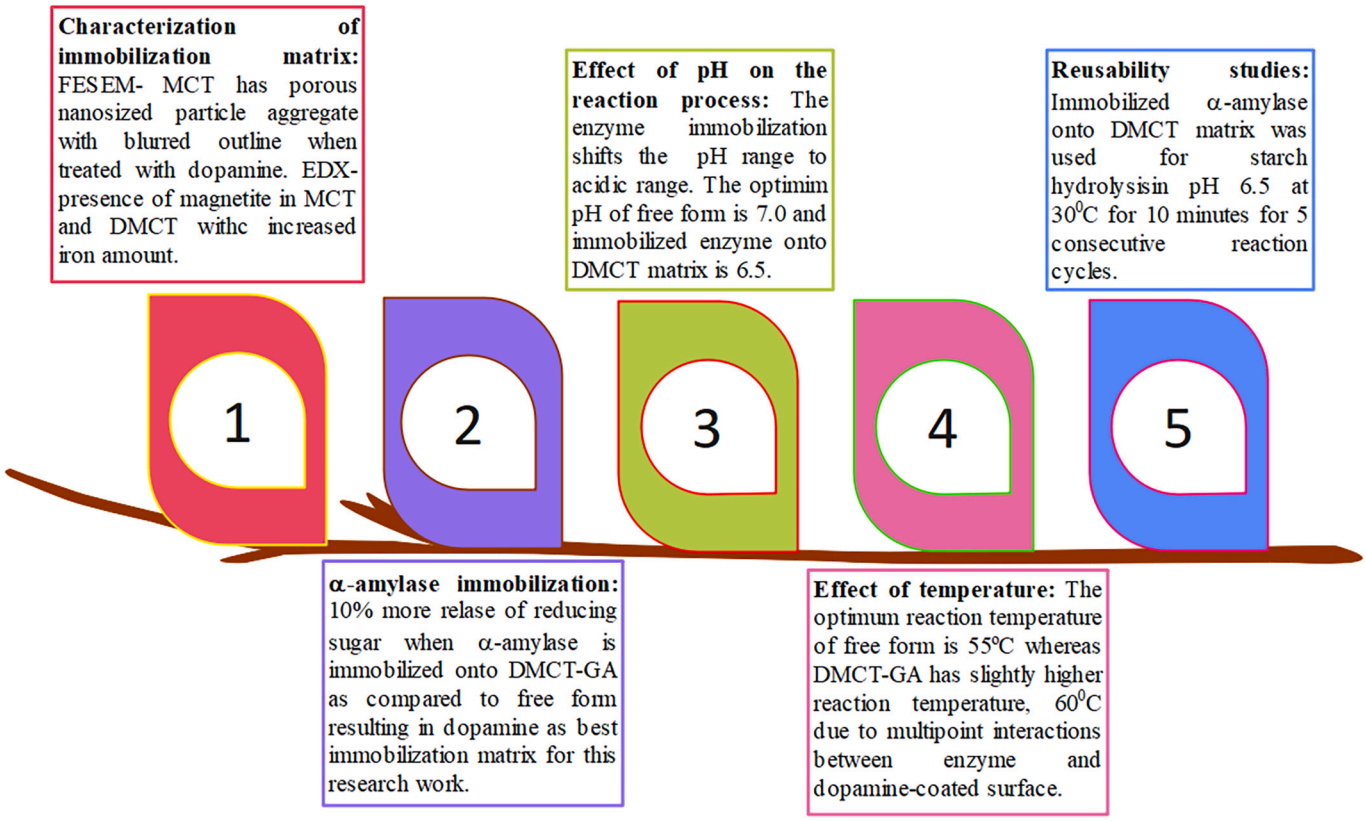

Fig. 6. Brief discussion of polydopamine-coated MCT as a matrix for enzyme immobilization [299].

glutaraldehyde. In other biotechnological applications, chitin particles could be utilized covered with polydopamine; its utilization can be presumed by the above examination [299].

4.2. A magnetic tri-enzyme nanobiocatalyst for the clarification of fruit juices

In the course of the foremost recent few years, the advancement of innovations for organic product juice preparation has pulled in impressive consideration for the better quality of juice due to increment in regular organic product utilization of juice [300]. The texture, color outlook alongside rheological conduct are the very important viewpoints to be observed while processing the fruit in a proportion value, yet additionally to assess the performances of procedures and equipment [301]. Due to the colloidal scattering of pectin (0.9-1.5%) present within the sorts of the disrupted cell membrane and cell materials of natural product, it overcasts in the appearance of the freshly squeezed turbid fruit juice i.e. the major obstacle in handling clear fruit juice [302]. Another potential supporter of the cloudiness of juice is starch and gel formation, hence causing trouble in the filtration. Moreover, the presence of various polysaccharides, for instance, the propensity of hemicellulose and cellulose to settle while storage bringing about clarity, low-quality fruit crush, and flavorless serum [303,304]. Right now, clarification processes for the juice density, ultrafiltration, and microfiltration are utilized. In any case, such filter advancements are restricted by many obstacles that show up while the expulsion of squeezed particles left in the solution creates membrane fouling and hence abbreviates the anticipation of membranes [301]. Along these lines, purification of juice should be completed before commercialization and filtration [305].

To maintain a strategic distance from undesirable shadiness alongside improving juice yield, storage stability, quality, first-rate, and garage stability, the utilization of enzymes has been expanded altogether in fruit juice manufacturing units. The most significant enzyme that is pectinase is fundamentally utilized in the purification of juice. By α-amylase, the lumps of pectin and starch molecules with proteins can be disposed of and along these lines nullifies murkiness [306]. Despite any such preferences of enzymes, the mechanical application is continuously hampered because of positive disadvantages, for example, absence of long-time firmness under working state, difficult recovery, and re-utilization of the enzymes [307]. To check such impediments, innovation regarding immobilization is contemplated as a major device for the betterment of the protein reactive characteristics regarding heat stability, resistance in the context of natural solvents, and high pH. Additionally, it reinforces numerous employments of highly-priced proteins providing monetary feasibility [308]. Since many previous years, in different immobilization systems, nanoparticles had been utilized as strong transporters. Additionally, appealing nanoparticles give excessive surface territory clean detachment and ease of reusability for various cycles [309].

The significant complexities in natural product juice satisfactory development are the presence of glycans additives as disrupted fruit cells and cellular substances. The disintegration of cellulose alongside starch and pectin is significant for the preparation of juice. In this precise circumstance, magnetic tri-enzyme nanobiocatalyst was set up by way of all the at the same time as co-immobilizing 3 catalysts; α-amylase, pectinase, and cellulase onto amino-functionalized magnetic nanoparticle through 60 mM glutaraldehyde concentration with 10 hours interlinking time for clarification of one container of juice. The warm (50-70°C) and pH (3-6) steadiness examination showed more than one folds increase down the middle life and improved resistance to convey down pH. The proteins that are immobilized are held as much as 75% of the lingering movement substantially after eight non-stop patterns of reuse [310] (Fig. 7).

The proteins that are immobilized are likewise showing better stability even at low pH ranging from 3-6. The K_m values were seen as

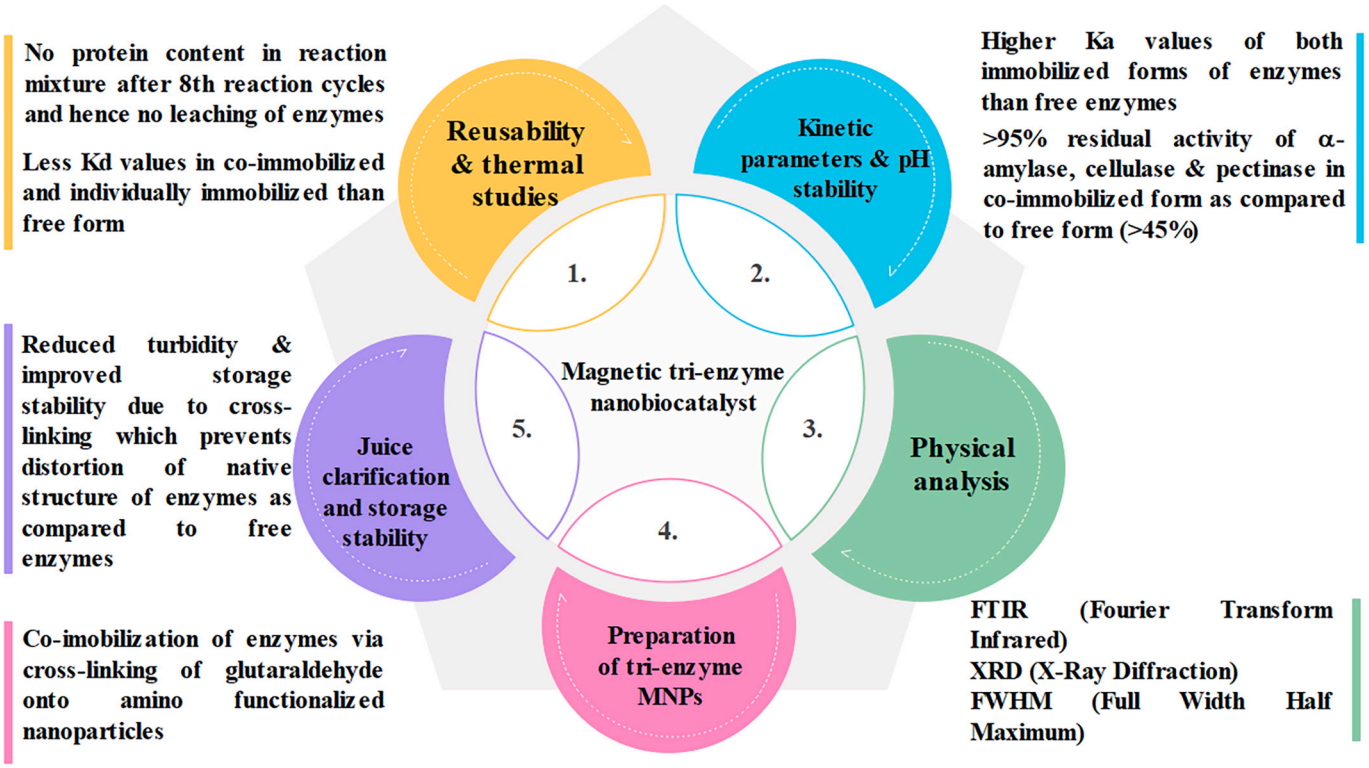

Fig. 7. Brief experimental set-up and discussion of magnetic tri-enzyme nano biocatalyst for fruit juice clarification [310].

higher though V_{max} values have been reduced for cellulase, α-amylase, and pectinase in immobilized structures. Hence, co-immobilized magnetic biocatalyst utilized for purification of apple, grapes, and pineapple juices exhibited a fast decrease to the extent of 46, 41, and 53% of their turbid nature separately up to the treatment of 150 minutes i.e., superior to blend of the structure of immobilization. These outcomes recommended that tri-enzyme magnetic nanobiocatalyst ought to be a possible option for customary natural product juice preparation in businesses [310].

4.3. *Bioactive flake–shell capsules for efficient enzyme immobilization*

Numerous utilizations of enzymes, and proteins by and enormous, include their associations with strong materials [311,312]. Protein immobilization is probably the simplest technique for the development of their working applications [313], it is due to rehashed use, increased stability, effortless partition from the response blend, and simpler item recuperation without enzyme defilement [314,315].

The most alluring backings for immobilization of protein are inorganic substances of which silica-based substances are of better appropriateness inferable from their great chemical resistance to organic solvent, better structural stability, sand microbial assaults, and natural balance, even as their low cytotoxicity and better biocompatibility [316]. Mesoporous silica nanoparticles (MSNs) are used widely for protein immobilization [317]. Nonetheless, the dimension dispersion of tiny holes in MSNs confines choice and amount of the stacking proteins [318]. Expanding the pore size favors protein stacking however prompts an expansion in protein desorption from molecules while re-scattering in solution. This component is a problem frequently happening in mesoporous silica particles [319]. In this manner, the creation of the latest silica shapes to upgrade the immobilization of protein is a huge test.

In such experiments, for improved immobilization of protein, we test silica capsules with flake shell morphology. Empty spheres or Capsules to be utilized as bio supports are the most valuable things [281]. Such structures offer not just help at their external surfaces and inside the pores yet additionally restrictions at their insides. Enzymes holding capsules are often utilized to perform natural procedures during a falsely bound condition that imitates biological reactors. Flake shell containers have an adaptable yet powerful shell and are created by hydrothermal utilizing strong silica particles as self-templating objects during their disintegration and moment re-development process [320].

The nonappearance of an arranged pore framework in the mass of the flake shell particles considers easily becoming a member of atoms of various sizes. These silica flake shell pills have vast superficial regions and capacity of holes, are made from a free flake sheet system, and consolidate the worthwhile properties of delicate polymeric capsules with the top-notch mechanical features of latent silica substances creating them remarkable up-and-comers as it supports productive enzyme immobilization [320]. The working of capsules with the ordinary polysaccharide dextran or amine groups turned into an investigation to assess the effect on the outer layer of both the capsules and their immobilization of protein capacities. To symbolize the extensive pertinence of the flake shell instances for immobilization of protein, we have determined the adsorption and movement for three normal generally taking place proteins of numerous, measurements, isoelectric and molecular weights focus chymotrypsin, lysozyme, and lipase [320].

From the trial data's, flake shell capsule A-C via warming antecedent strong silica particles at 75°C in an autoclave for 24 hours; D has been framed with the aid of oppressing robust silica circles; A to APTES surface alteration in dry toluene for twenty-four hours; E to dextran surface corporations by coupling CM-Dextran to the amino-modified molecules via H_2O dissolvable carbodiimide EDC at 4° C for forty-eight hours. Two kinds of surface-modified silica particles had been utilized to ponder the effect of surface functionalization on the shell shape of the capsule. Capsules B was obtained from amino-modified silica molecules, and capsules C had been shaped from dextran-altered particles. To discover the protein take-up limits of flake shell silica capsules, 3 model proteins of diverse molecular weights, measurements, and isoelectric points (pI) had been applied. Lysozyme was selected as a run-of-the-mill acidic protein, little globular (3 nm and MW= 14 kDa, pI 11.3). Also, lipase

becomes picked as an enormous, neutral protein (14 nm, MW= forty kDa, pI 6.3), and chymotrypsin became picked as a protein with neutral conditions (7 nm, MW= 25 kDa, pI 8.7). H2O molecules suspensions (1 mg/mL) had been presented to protein solution (1 mg/mL) in PBS buffer (pH 7.4, 24 hours). Superior stacking of proteins on flake shell containers contrasted with robust silica spheres changed into watching. It has been seen from the examinations that both the surface chemistry and shell structure may impact the protein take-up no matter the fact that changes of these factors didn't improve enzyme stacking Immobilization of enzymes frequently prompts a decrease in action [40] thus, the held action of an adsorbed enzyme (per gram protein) is a significant pointer of the successful immobilization of enzyme. The progressions in synergist action of enzymes that are immobilized contrasted with the free enzyme in the liquid mixture have not happened just by variations in their intrinsic activity micro-habitat in which the protein molecules interactions happen, the vague connections of enzyme-substrate support, and hindrances in chemical reactions steric obstacles for macromolecular substrates. The constrained access of substrate molecules to proteins that have been entangled among the flake or inner side of the flake shell capsules precipitated the observed decline in enzymatic action contrasted with the loose enzyme in the liquid mixture (Fig. 8) [320].

As shown in the usage of flake shell silica capsules as platforms for effective enzyme immobilization. The huge surface territories pore volumes and empty superficial territories of those molecules empowered generous stacking of macro-particles. Lower desorption of the enzymes that are immobilized, and dissemination of micro-molecules preferring quicker movements. Aside from lysozyme movement in the lysing of cells, in which the enzymes had restricted access to its molecules of substrate, the obvious synergist movement of the proteins became all round held after immobilization at the flake shell capsules. Furthermore, the flake shell silica capsules demonstrated mild degradation under low cytotoxicity and physiological conditions, when applied for polyethyleneimine covering and stacking of CpG oligodeoxynucleotides [321]. Along these lines, such capsules are suitable as supports in different biological uses. These changes inside the shell structure and chemistry impacted the stacking of proteins and were demonstrated to be valuable for the manipulation of their bioactivity. Hence, the shell morphology, composition, and structure of the flake shell silica capsules advance biocompatibility, better enzyme loading, mechanical stability, biologically active, creating reasonably for a better scope of uses, for example, in drug delivery, bio-sensor, bioactivity, biocatalysis, and encapsulation [320].

4.4. Encapsulating chloroperoxidase (CPO) in hybrid polysaccharide-silica biocomposites

Protein catalysis in natural aggregate is generally perceived as likely the primary decision within the association of a wide scope of chemical compounds, for scholastic synthesis, yet further for mechanical scale programs. In any case, the work of catalysts of their local structure for contemporary uses is regularly impeded by way of large expenses, lower working stability, and challenges in recovery and re-utilization of the bio-synergies. Immobilization of enzymes onto robust backings speaks to certainly one of the most alluring strategies to defeat these downsides [51]. An assortment of engineered organic or inorganic polymers and biopolymers may be applied as supports, and proteins may be immobilized through physical adsorption, covalent binding on the carrier surface, interlinking, or encapsulation. The help and the technique for immobilization are of central importance for an effective huge scope use of biocatalysts, since the real procedure utilized for every enzyme has to be painstakingly picked and relies upon the selected enzyme [322].

Chloroperoxidase (CPO) from *Caldariomyces fungo*, an intensely glycosylated heme enzyme, is an exceptionally adaptable enzyme that catalyzes distinctive types of reaction, a huge number of which can be of current intrigue that is, sulfoxidation, hydroxylation, and epoxidation [323]. Despite the reality that these points of interest, deactivation of the enzyme at excessive heating and inside the sight of oxidizing agents which were concentrated, restrains the CPO engineered uses. Various endeavors have been coordinated to improvise CPO reactant characteristics, the least hard among them being the utilization of added materials in the aqueous mixture [322].

Covalent connection on chitosan membranes [324], on an amino-agarose gel [325], on methacrylate and magnetic beads [326], aminopropyl-glass [327], and mesoporous silica [328,329] typically involved the development of covalent bonds among the carboxyl groups or carboxyl groups at the enzyme's superficial region and presence of dynamic region support. For this situation, leakage of protein is frequently confined at the same time, due to the binding response, lost enzymatic action can be seen because of connecting parameters (temperature, pH, medium, added substances), to an expansion in proteins unbending nature or steric obstruction of the active surface [322].

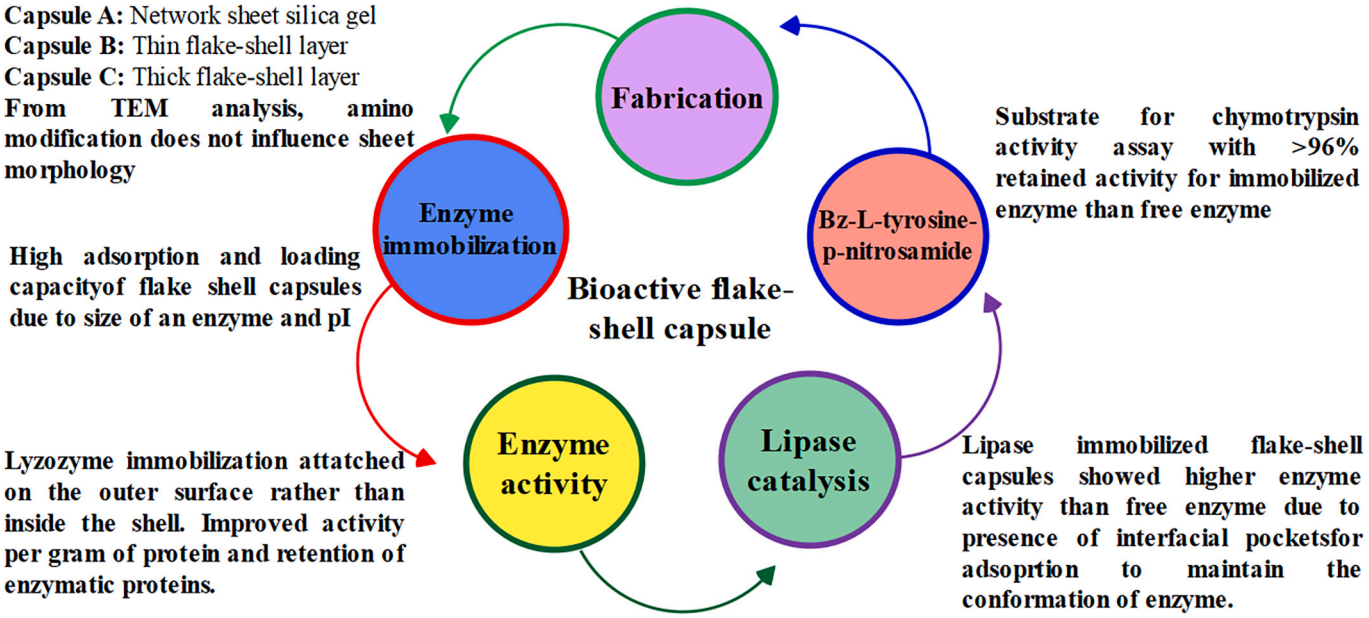

Fig. 8. Brief representation of bioactive flake-shell capsules for effective enzyme immobilization [320].

On this point, we record results received after the biocatalyst effectiveness and CPO encapsulation into hybrid glycan-silica lattices in a reaction of chlorination has been researched. Encapsulation of CPO molecules into a lattice-shaped in situ by reaction of polycondensation. The substrates of characteristics relied upon the kind of glycans utilized on their charge and concentration. Two direct glycans, chitosan and sodium alginate these contrasts in its practical gatherings, for example, carboxyl and amino individually, had been chosen. The basic portrayal of half-breed silicates was cultivated with the aid of SEM and the impact of the glycan-silica helps on CPO productivity, re-utilization, and heat stability were assessed. Polysaccharides communicate to accessing agents for doping to work with nanoparticles of silica and their excessive similarity with proteins creates these half-breed materials for immobilization of enzymes [322].

Two extend polymers, that is a heteropolysaccharide, a homoglycan of glucose, dextran, and xanthan, formed by way of glucuronic acid, glucose, mannose, and chitosan, made out haphazardly by distributing of N-acetyl-d-glucosamine, D-glucosamine, and its C-5 epimer l-guluronate a linear anionic copolymer of D-mannuronate, and sodium alginate, are chosen to examine their impact on the characteristics of a biological synergist. Polysaccharides can catalyze the sol-gel process and in their presence, gelatin maintains even at pH esteems at which generally applied antecedents don't shape gels. Within the sight of all of the chosen delivered substances, no leaching of the enzyme was seen within the cleaning solutions and in this way, the shape of the doping agents and their connections with the pore silica and the enzyme surface had been essential to creating a proficient biological synergist. The balancing effect of glycans on the captured CPO is regarded to be reliant on the direct or expanded characteristic of the polymer. Spread polymers most probably created a very close system that would restrict the conformational freedom of entrapped protein, or prevent an affordable diffusion of the substrate, prompting a wasteful biological synergist [322].

To verify the constructive outcome of chitosan amino businesses and to take a look at the job of the polymeric shape of the introduced substance on immobilized CPO re-utilization, chitosan monomer, D-glucosamine is utilized as an added substance in silica hybrid composites. D-glucosamine is a better aqueous soluble compound so it permitted planning half breed or hybrid silica matrices with altogether different added substances, up to 2 wt%. By using the broadness of the concentration range, at all the examined added substance concentrations, the time of gelation is around 4 hours. Water content is a condition that can assume a significant task on conclusive biocatalyst effectiveness because it could affect the structure of the matrix [322].

The key characteristic of the biocatalyst is thermal stability particularly in the context of uses in mechanical bioconversion from the likelihood to upgrade their opposition towards upper temp. would offer clear advantages to functional uses. The heat stability of immobilized CPO was at that factor explored with the covalent immobilization of a protein on totally acrylate-based magnet beads and onto adsorbed or covalently connected in SBA-16 mesoporous materials [328], p (HPMA)-co-PEG-MA membranes [236] and temp equal or more than sixty-degree Celsius, protein total loss in no time flat [322].

Immobilization onto sturdy backings is a dazzling methodology to enhance protein catalysis ability and route to their uses in scholarly formation with the mechanical advent of chemicals. Significant difficulties inside the developing modern-day biocatalysts for mechanical software are spoken to by way of advanced strength, mainly as a way of protection from high temp and denaturants, and through simpler recovery and re-utilization. Specifically, silica-chitosan parts are visible as the best backings to enhance biological synergies re-utilization. Basic parameters at first received, protein executed five whole cycles and changed over to 60% of the desired substrate at 6th cycles. The exploratory method was then transformed (i) through dividing the reaction time, and afterward the oxidation pressure, (ii) by way of removing the purification of lattices among the chemical cycles, therefore, diminishing the mechanical stress because of centrifugation, and (iii) by enhancing the concentration of chitosan in lattices. In these conditions, at the most elevated workable achievable concentration, the biological synergist had the choice to catalyze the conversion of substrate to the extent of 18 reaction cycles, improving CPO recyclability. Silica-chitosan halfbrid lattices likewise shielded CPO's deactivating effect of high temp: the free enzyme was losing approximately half of its action in a few minutes at 60 and 70°C, while, at comparable temperatures, the enzyme action stayed better than 95% significantly following two hours. Outcomes that had accomplished this work on the CPO results are extremely encouraging taking into consideration engineered utilization of this flexible catalyst (Fig. 9) [322].

5. The challenges associated with polysaccharide-based enzyme immobilization scrutinization

Various endeavors have brought about progressions in the field of immobilization of protein utilizing glycans for different uses. With no equivocalness, such various uses have encouraged us to exploit enzymes appropriately to chop down the related expenses through successful recycling and legitimate procedure. Previously, the procedure immobilization was significantly engaged to balance out and reuse hardware for systematic and clinical applications, for instance, (a) picking explicit adsorbents for enzyme and protein purification, (b) operative microequipment to appropriately manage the release of protein-based medications, and (c) noteworthy gadget for solid face chemistry. The headways in the field of enzyme immobilization have encouraged scientists to actualize protein and cell framework as far as biocatalyst in various mechanical applications, for example, the field of fine chemistry, analytical chemistry, food chemistry cleaning procedures, and remedial applications. It has consistently been good to have better properties of mechanical-based immobilized enzymes, for example, action, selectivity, soundness, cost-viability, security, development, and so forth. Consequently, it is strongly prescribed to focus on the advancement of bio-immobilization and consider it as a novel strategy for immobilization of protein that completes the techniques of carrier-free and carrier bound aspects with better stacking of enzymes, better arrangement of reactors, upgraded action maintenance, and so forth. Presently, the way to deal with such measures is very uncommon and henceforth the significant concentration in close to prospects depends on the improvement of polysaccharide-based bearers with suitable chemical and physical properties to straightforwardly hold enzymes under mellow conditions. Besides, the expansion in various immobilization approaches in the coming future will consequently help us in solving the issues related to the single immobilization techniques. Also, a superior comprehension of both enzyme selectivity and the structural alterations and bridging the same because of genetic engineering and other chemical changes have heightened the consideration in upgrading the selectivity of enzymes through immobilization [91].

6. Conclusion

With globalization in the field of enzyme immobilization, it has become a promising strategy to enhance the efficiency and economy of various biotechnological applications such as environmental monitoring, biotransformation, diagnostics, pharmaceutics, chemical industries, and food industries. Enzyme-based approaches are rapidly replacing traditional chemical methods, at laboratory scale and industrial scale, respectively with promising characteristics such as improved efficacy, rapid performance, and diversified usage. Nevertheless, the commercialization of immobilized enzymes is at a slower pace due to high costs and storage issues. The utilization of varied types of carriers ensures better reusability and recoverability of enzymes resulting in a decrease in operational costs. Additionally, owing to the protective nature of the matrix, immobilized enzymes have become resistant to alterations in environmental parameters like temperature, pH, thermal

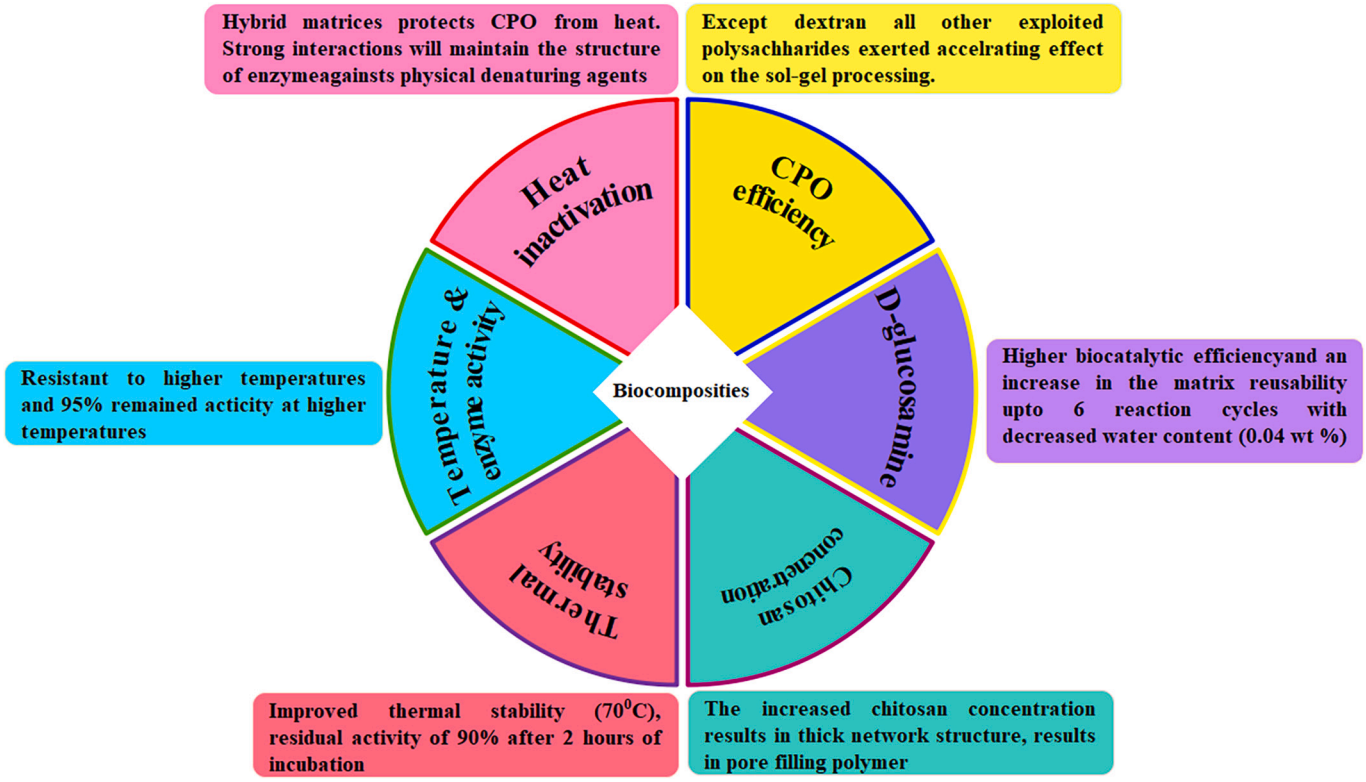

Fig. 9. An outline of CPO-based hybrid polysaccharides-silica biocomposites [322].

stability, storage stability, chemical denaturation, the inhibitory effect of various compounds, etc. This ultimately results in better operational stability of immobilized enzymes. There is a dire need to focus on overcoming existing limitations of enzyme immobilization approach intending to broaden the horizons of enzyme immobilized-based industrial applications. This review discusses how the usage of polysaccharides in conjugation with enzyme immobilization approaches have improved the action and soundness of enzymes in the different reaction environment. Polysaccharides are a type of carbohydrates that are formed from >10 to 1000s of monosaccharides (glucose, fructose, galactose, and mannose) which are connected via glycosidic bonds. All over the globe, over the years, the demand for polysaccharides has accelerated with significant exploitation in diverse sectors such as food industries, chemical industries, biosensors, etc. Presently, researchers are putting extensive efforts into the domain of enzyme immobilization by utilizing polysaccharides as base supports for numerous purposes. Undoubtedly, such applications have suitably utilized enzymes intending to diminish the cost of production via effective recycling and controllable process. With a rare scope of meeting certain requirements such as high loading of enzymes, broad configuration of reactors, improved retention activity of enzymes, the major focus lies on developing polysaccharide base supports with appropriate chemical and physical characteristic properties that have significant potential of holding enzymes under mild reaction conditions. With careful examination and appropriate arrangements as the support network, the polysaccharides have proved to be a potential candidate in enzyme immobilization, for example, the use of nanoparticles into the matrix of polysaccharides, nano biocomposites of glycan lattice, has better adaptability of being transporter with the better rigidity of enzymes because of their minute structure and enormous superficial area. Furthermore, the immobilized enzyme onto nanoparticles covered with a polysaccharide matrix has expanded the reliance and portability of enzymes with improved biocompatibility and biodegradability. Also, with advancement in the field of genetic engineering, there has been an immense increase in understanding the connection between the selectivity of enzymes and alterations in the structure of the enzyme. The future progress is inclined much more to improving the selectivity of enzymes via immobilization techniques.

Declaration of Competing Interest

The authors with listed names declare no conflict of interest to disclose.

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