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Historical Perspective

Alginate-based materials for enzyme encapsulation

Yilun Weng [a], Guangze Yang [b], Yang Li [a], Letao Xu [a], Xiaojing Chen [c], Hao Song [a], Chun-Xia Zhao [a,b,*]

[a] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, QLD 4072, Australia
[b] School of Chemical Engineering, The University of Adelaide, Adelaide, SA 5005, Australia
[c] Bioproton Pty Ltd., Brisbane, QLD 4110, Australia

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ABSTRACT

Enzymes are widely used in industry due to their high efficiency and selectivity. However, their low stability during certain industrial processes can result in a significant loss of catalytic activity. Encapsulation is a promising technique that can stabilize enzymes by protecting them from environmental stresses such as extreme temperature and pH, mechanical force, organic solvents, and proteases. Alginate and alginate-based materials have emerged as effective carriers for enzyme encapsulation due to their biocompatibility, biodegradability, and ability to form gel beads through ionic gelation. This review presents various alginate-based encapsulation systems for enzyme stabilization and explores their applications in different industries. We discuss the preparation methods of alginate encapsulated enzymes and analyze the release mechanisms of enzymes from alginate materials. Additionally, we summarize the characterization techniques used for enzyme-alginate composites. This review provides insights into the use of alginate encapsulation as a means of stabilizing enzymes and highlights the potential benefits for various industrial applications.

1. Introduction

Enzymes are macromolecular biocatalysts that have been widely used in various industries due to their efficiency, selectivity, and environmental sustainability [1]. However, their industrial applications are significantly hindered by their instability [2]. When enzymes are exposed to unfavorable conditions in industrial processes, such as elevated temperature and altered pH, their activity is often compromised [2,3]. To overcome this issue, several strategies have been developed to stabilize enzymes, including gene discovery [4], protein engineering [5], and encapsulation [6]. Enzyme encapsulation has emerged as a promising approach for stabilizing enzymes by protecting them from harsh environmental conditions. This review focuses on the encapsulation of enzymes which has demonstrated great promise for various industrial applications.

Enzyme encapsulation involves confining enzymes within a matrix (the coating/support material) to minimize structural changes and prevent enzyme degradation [7]. Encapsulated enzymes are more stable under various conditions than their native forms. Furthermore, encapsulation can enhance enzymatic activity by altering hydrophobic interactions [8], increasing reaction surface area [9], and increasing the local concentration of intermediates [10]. As a result, encapsulated enzymes have been widely used in various industries, including biocatalysis, biosensing, enzyme therapy, biomedicine, and bioremediation [11,12].

Enzyme encapsulation can be achieved through the use of various coating materials, such as organic, inorganic, and organic-inorganic hybrid materials. Among these options, natural polymers are

Abbreviations: AFM, atomic force microscopy; AKTA, ÄKTA chromatography; CAT, catalase; CD, circular dichroism; CLA, colloidal liquid aphron; CLSM, confocal laser scanning microscopy; Cy 3, cyanine 3; Cy 5, cyanine 5; DLS, dynamic light scattering; DSC, differential scanning calorimetry; EE, encapsulation efficiency; FITC, fluorescein isothiocyanate; FTIR, Fourier transform infrared spectroscopy; GIT, gastrointestinal tract; GOx, glucose oxidase; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; LC-MS, liquid chromatography–mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; OVA, ovalbumin; PCL, polycaprolactone; PDA, polydopamine; PEG, polyethylene glycol; PEI, poly(ethyleneimine); PLA, polylactic acid; PLGA, poly(lactic-*co*-glycolic acid); PVA, polyvinyl alcohol; PVC, poly(vinyl chloride); RS, Raman spectroscopy; SAXS, small-angle X-ray scattering; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SEE, single enzyme encapsulation; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TGA, thermal gravimetric analysis; UV–Vis, ultraviolet-visible spectroscopy; XPS, X-ray photoelectron spectroscopy; XRD, X-ray powder diffraction; YADH, yeast alcohol dehydrogenase.

* Corresponding author at: School of Chemical Engineering, The University of Adelaide, Adelaide, SA 5005, Australia
E-mail address: chunxia.zhao@adelaide.edu.au (C.-X. Zhao).

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particularly popular due to their excellent bioavailability, non-toxicity, and low cost [13]. One type of natural polymers that has received significant attention in the field of material science is alginate-based materials, which are known for their versatility and biocompatibility [14]. Over the past decade, alginate has been extensively studied as an encapsulation material, with the number of publications on the topic still increasing (Fig. 1). Alginate is a linear polysaccharide composed of beta-D-mannuronic acid (M) and alpha-L-guluronic acid (G) residues that can be found in the cell walls of brown seaweeds (Fig. 2A) [15]. The M and G residues can be arranged in different ratios, resulting in the formation of homo-polymeric G-blocks, homo-polymeric M blocks, and hetero-polymeric MG alternating blocks (Fig. 2B). These different structures provide options for the rational design of alginate-based encapsulation systems with different cargo loading capacities and release profiles [16]. One of the unique properties of alginate is their ability to form gels in the presence of divalent cations such as Ca^{2+} and Ba^{2+} [17]. This property, known as gelation, results from the ionotropic crosslinking of alginate chains. The properties of alginate-based materials can be tailored for specific applications by adjusting their chemical composition, molecular weight, and gelation conditions.

This review begins by introducing various types of alginate-based encapsulation systems for enzyme encapsulation and their fabrication methods. It then explores common characterization techniques as well as the release mechanism of enzyme-loaded alginate materials. Finally, the review summarizes the relevant industrial applications of alginate in enzyme encapsulation. This review provides a comprehensive overview of how the physicochemical properties of alginate relate to its applications in enzyme encapsulation for various industrial applications.

2. Alginate-based encapsulation systems

Encapsulation using alginate-based materials is promising for stabilizing bioactive ingredients, such as enzymes and drugs. Alginate is biocompatible, biodegradable, and low-toxicity polysaccharides derived from seaweed. It can be used to create hydrogels [18]. Encapsulating enzymes or drugs within these hydrogels can protect them from degradation, improve their stability and potentially target them to specific sites within the body.

Alginate composites can take various forms, including alginate spheres (gel beads and emulsions), alginate films, and alginate fibers (Fig. 3A) [16]. Despite their differing shapes, the major distinction between these forms of alginate composites lies in their degree of gelation, which is determined by the different precursor concentrations (*e.g.* sodium alginate and calcium ions) used during synthesis [19]. Generally, alginate films require the lowest precursor concentrations, followed by alginate fibers, while alginate beads necessitate the highlight level of alginate and calcium concentrations. This results in various levels of structural flexibility and stiffness of these alginate composites, each with distinct applications. Alginate spheres are widely used for enzyme encapsulation due to their excellent stability and simple synthesis. Alginate films are primarily employed for food packaging and preservation, where anti-bacterial enzymes like lysozyme can be encapsulated to enhance their activity [20]. Alginate fibers have also been utilized for enzyme encapsulation, but current research focuses more on developing biomedical materials for tissue engineering and wound dressing due to their good biocompatibility and high mechanical stability [19]. Furthermore, alginate and its composites can be modified with different functional groups to adjust their properties, such as release rate, biocompatibility, and swelling behavior [21].

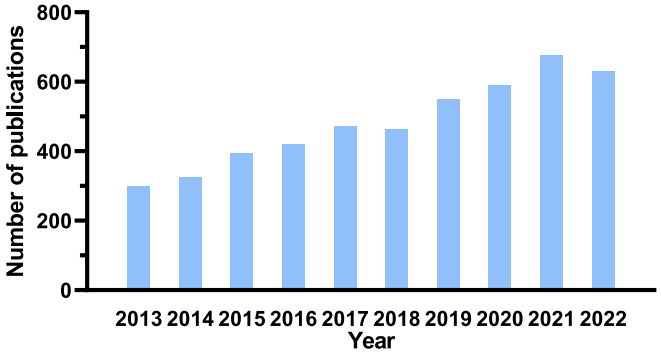

Fig. 1. Number of publications of encapsulation studies using alginate in the last decade, obtained from Web of Science database (https://www.webofscience.com, accessed Jan. 15, 2023).

2.1. Alginate spheres

Alginate gels have a highly hydrated and porous structure, making them attractive for a variety of biomedical applications including drug delivery, tissue engineering, and wound healing [22]. Among the different forms of alginate, gel beads are the most commonly used form for the encapsulation and delivery of enzymes due to the simple preparation procedures and mild conditions. Typically, dripping alginate droplets into solutions containing divalent ions to form gel beads through ionic gelation is one of the most widely used methods for the encapsulation of enzymes and other bioactive ingredients [23]. Ideally, the properties of alginate gel beads such as beads size, porosity, and release profile can be controlled by tuning factors such as pH [24] and alginate precursor concentrations [25].

Despite the simple synthesis method and its capability in stabilizing enzymes, the alginate beads-based encapsulation approach often suffers from low encapsulation efficiency, enzyme leaching, and unsatisfactory enzyme recycling [26]. To address these issues, an additional layer of coating outside the alginate beads can be employed. For instance, Pauly et al. significantly enhanced the activity and encapsulation efficiency of pig liver esterase *via* a chitosan coating [26], while Rehbein et al. improved the structural and mechanical stability of encapsulated recombinant enzymes using silica-coated alginate beads [27]. In another study, the microstructure stability of lactase in Ca-alginate beads against freezing and dehydration was improved with the addition of trehalose and gums [28]. The incorporation of gums as the second excipient significantly improved the microstructure stability of the enzyme-alginate composite, at both the rod scale, and the characteristic size and density at a larger scale, thus enhancing the enzyme stability. Moreover, the encapsulation efficiency can also be improved by controlling the concentrations of both alginate and the divalent ions [29].

Another type of alginate spheres can be fabricated based on alginate-in-oil emulsions. An emulsion is a mixture of two immiscible liquids. For example, water-in-oil emulsions are water droplets dispersed in an oil phase that are formed through an energy input [30]. Due to the different solubilities of bioactive ingredients in water and oil, emulsion-based delivery systems have been developed [31]. To date, various emulsion systems have been designed to improve oxidative stability and enhance the protection of the encapsulated bioactive ingredients. Alginate-based emulsion systems are attractive for the encapsulation, stabilization, and controlled release of enzymes. Two most common types of emulsions for the encapsulation and delivery of bioactives are oil-in-water (O/W) emulsion and water-in-oil (W/O) emulsion. Baimark and Srisuwan prepared alginate microspheres for the encapsulation of blue dextran using the water-in-oil emulsion method with a high encapsulation efficiency (82%) [32]. A prolonged drug release profile was observed when the alginate microsphere was crosslinked with Ca^{2+}. In another study, Singh et al. prepared a multi-functional oil-in-water Pickering emulsion with improved oxidative stability, pH-responsiveness, and mucoadhesiveness using chitosan and alginate, which showed great potential for food applications [33].

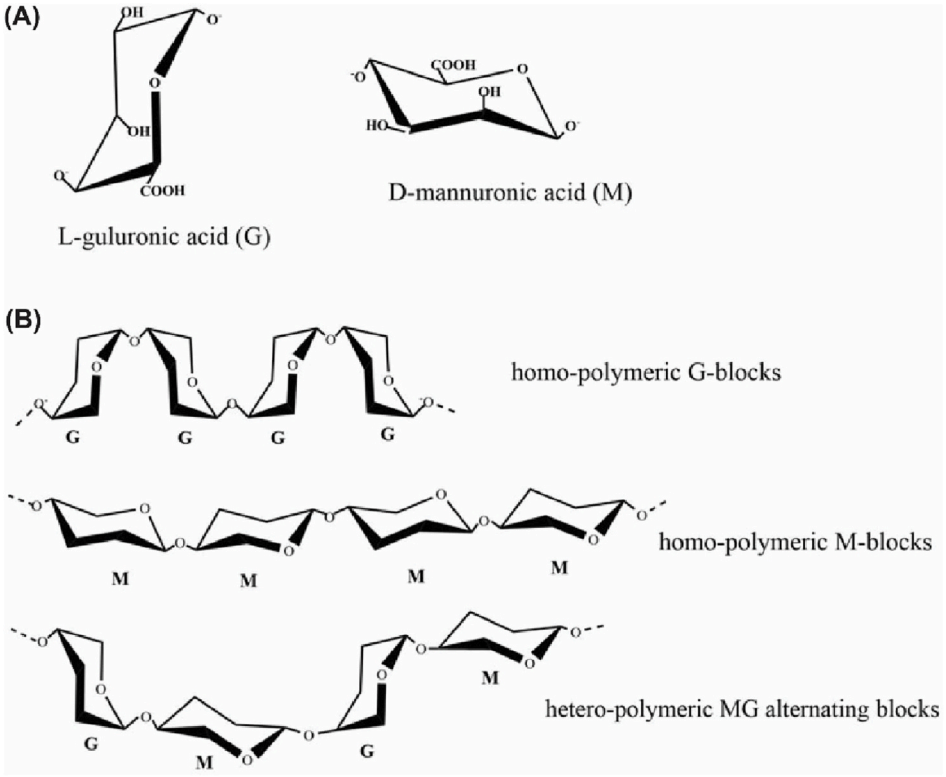

Fig. 2. Alginate chemical structure: (A) alginate monomers: L-guluronic acid (G) and D-mannuronic acid (M), (B) homo-polymeric G-blocks, homo-polymeric M-blocks and hetero-polymeric MG alternating blocks. Adapted from [16] with permission.

2.2. Alginate film

Alginate film is also attractive for biomaterial encapsulation due to its gelation properties and fibrous chemical structure [34]. In food and pharmaceutical industry, alginate-based films have been used for encapsulating bioactive components, due to their versatility, uniformity, transparency, water solubility, biocompatibility, biodegradability, and economy [35]. Edible alginate films are mostly applied in food industry for food packaging and preservation due to their physical shape and non-toxicity [36]. Recently, Wang et al. successfully immobilized lysozyme into alginate films for antimicrobial applications, and the maximum enzyme catalytic activity was increased by 13.2% after immobilization [20]. Jiang et al. encapsulated yeast alcohol dehydrogenase (YADH) into alginate-chitosan films, and significantly improved enzyme stability including thermal stability, recycling stability, and storage stability [37]. Compared to free YADH, the encapsulated enzyme showed a 25% increase of activity at 50 °C, a 35% increase of activity after 10 recycles, and a 60% increase of stability after 10 storage days. Despite the great stability improvement, the circular dichroism (CD) spectra revealed that the encapsulation process did not affect enzyme activity, as the conformation of YADH after encapsulation was similar to that of free YADH. Additionally, alginate film was used for the encapsulation and delivery of Rv-Soy protein for colorectal cancer treatment [38], and the controlled release of drug was attributed to the pH responsive alginate.

2.3. Alginate fiber

The use of alginate fiber as encapsulation materials has attracted extensive interest due to its large surface area, high mechanical stability, tunable properties, and easy handling. Recently, Zhang et al. constructed alginate-based microfibers with tunable size, composition and degree of crosslinking using a home-made co-flow microfluidic chip [39]. Two enzymes, glucose oxidase (GOx), and horseradish peroxidase (HRP) were encapsulated into the alginate microfiber for glucose detection and the enzyme leakage was minimized by covalently grafting enzymes to poly(acrylic acid) (Fig. 3B, C). Compared to native enzymes, enzyme thermal stability was improved by 50% at 60 °C after encapsulation and over 85% of the activity was retained after seven cycles of reuse. In another recent study, alginate microfiber with tunable size, porosity, and surface topographies was synthesized for encapsulating cells to improve cell viability (Fig. 3D) [40]. Asthena et al. significantly improved enzyme thermal stability by encapsulating bromoperoxidase in alginate hollow fiber [41].

2.4. Modified alginate

Alginate-based materials can be modified through chemical methods to tailor their properties for specific applications [42]. As alginate has a number of free carboxyl and hydroxyl groups along its backbone, lots of chemical modifications can be applied to improve the material's biocompatibility and enzyme delivery capabilities. The introduction of new functional groups into alginate molecular skeleton as well as adjusting the existing functional groups can change the properties of alginate derivatives, such as hydrophobicity, solubility, and biological, chemical, and mechanical properties. For example, the hydrophobicity of alginate can be adjusted by changing the length of the alkyl chain, in order to enhance its affinity with hydrophobic bioactive ingredients [43]. Pelletier et al. demonstrated that the hydrophobicity of modified alginate was significantly improved with the increase of alkyl chain length [44]. Chemical modification techniques such as oxidation, sulfation, esterification, amidation, or grafting methods are commonly used [21]. The chemical modifications of hydroxyl groups mainly comprise oxidation, copolymerization, and sulfation, while the modifications of carboxyl groups mainly include esterification and amidation.

2.4.1. Modifications on hydroxyl groups

Oxidative modification of alginate is generally achieved by randomly oxidizing the hydroxyl groups of alginate molecules into carbonyl,

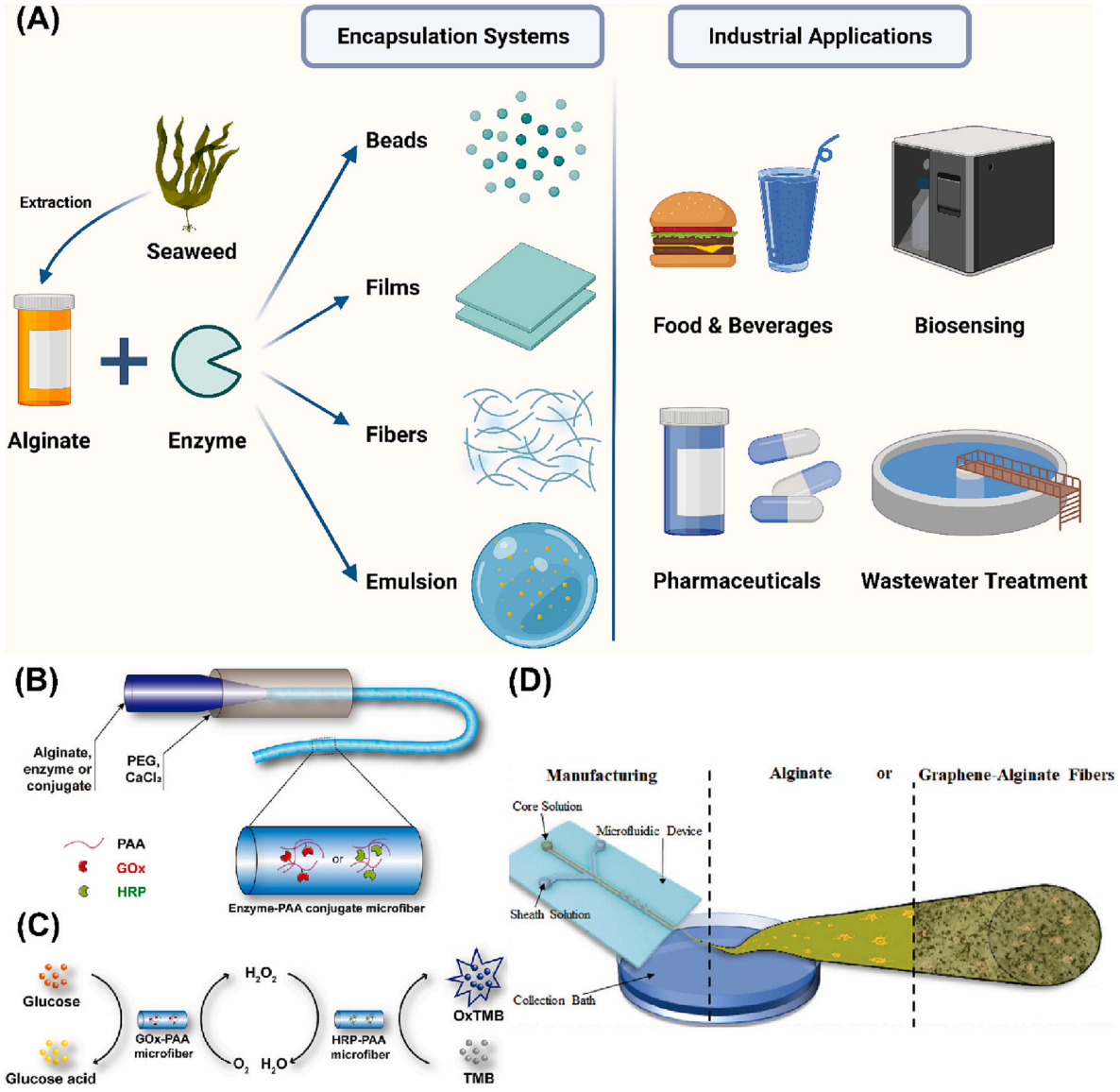

Fig. 3. (A) Alginate based encapsulation systems for enzyme encapsulation, and their industrial applications, (B) the procedure of encapsulating enzyme in alginate microfibers and (C) glucose detection in alginate microfibers, adapted from [39] with permission, and (D) the procedure of encapsulating enzyme in pure alginate or graphene-alginate microfibers using microfluidic fabrication. Adapted from [40] with permission.

aldehyde, or carboxyl groups using oxidizing agents (Fig. 4A) [45]. Two commonly used oxidizing agents are sodium hypochlorite and sodium periodate. However, due to the potential toxicity of these chemical reagents, the oxidizing agents must be carefully selected according to individual applications. Oxidation reactions on hydroxyl groups are mainly used to improve the biodegradability and decrease the stiffness of alginate [46,47]. For example, oxidized alginate contains more reactive functional groups compared with its natural form, resulting in a faster degradation rate. This can facilitate the controlled enzyme release [48]. Furthermore, the oxidation rate of the modified alginate needs to be controlled as a previous study showed that an oxidation rate greater than 10% could decrease the alginate gelling capacity, due to the disruption of the backbone structure [49].

The oxidized alginate contains aldehyde groups in its polymer chain, which enables further functionalization, such as reductive-amination. The reductive-amination of oxidized alginate (Fig. 4B) is employed to lower the surface tension and increase the pore dimensions and alginate biocompatibility, in order to improve the cargo loading capacity and enzyme release [50]. Recently. Chen et al. used the reductive-amination modification method to improve alginate affinity with hydrophobic ibuprofen [51]. The reductive-amination reaction broke alginate intramolecular hydrogen bond, improving the molecular flexibility and colloidal interface activity of the modified alginate.

Other chemical modifications on hydroxyl groups of alginate are sulfation (Fig. 4C) and copolymerization (Fig. 4D), which have found various applications in industry [52]. Sulfation modification of alginate refers to the substitution of the hydroxyl groups on the C-2 or C-3 carbon positions using sulfate groups. Sulfation can change the solubility of alginate by reducing the hydrogen bond density. Recently, Samsonchi et al. used the sulfation modification method to adjust the biocompatibility and anti-fibrotic property of alginate, making the sulfated alginate a suitable coating material for islet-containing microcapsules for the treatment of type 1 diabetes [53]. Copolymerization modification of alginate refers to the grafting of polymers onto the alginate main chain as branch chains. Copolymerization enables various functional properties of the modified alginate by grafting polymers with different structures and properties onto the main chain [45]. For example, Sand et al. grafted vinyl sulfonic acid onto alginate, which improved the thermal stability and water swelling capacity, while reduced the biodegradability of the synthesized graft copolymer [54].

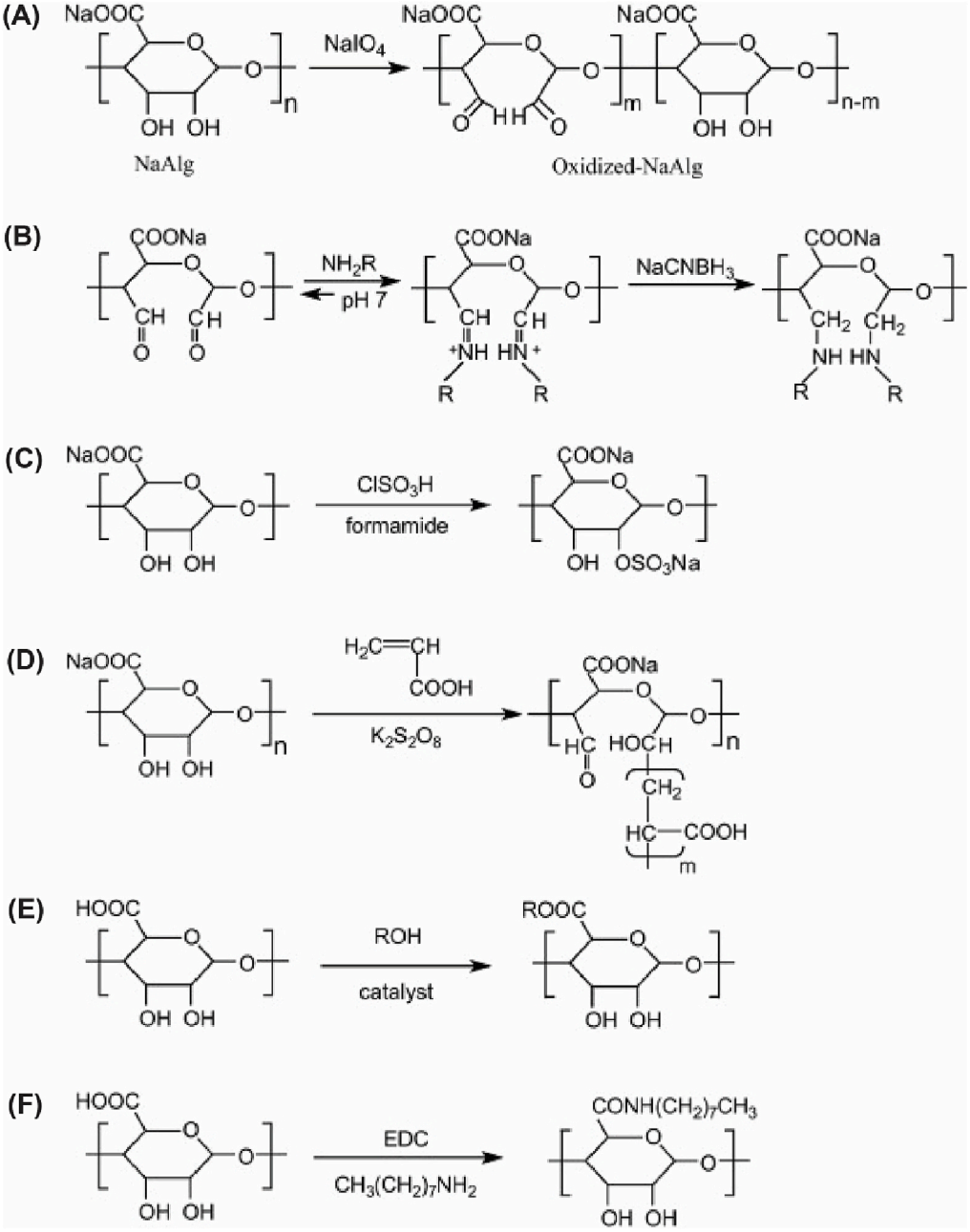

Fig. 4. Chemical modifications of alginate: (A) oxidation of hydroxyl group, (B) reductive-amination of oxidized alginate, (C) Sulfation of hydroxyl group, (D) copolymerization of hydroxyl group, (E) esterification of carboxyl group, and (F) amidation of carboxyl group. Adapted from [21] with permission.

2.4.2. Modifications on carboxyl groups

Esterification of alginate refers to the dehydration of its carboxyl groups to form ester bonds in the presence of a catalyst (Fig. 4E) [45]. The esterified alginate presents good encapsulation performances and has been applied as an emulsifier, thickener, and stabilizer in food industry [55]. Esterification of alginate can improve the biological activity of alginate, such as enhancing the antibacterial and antioxidant activity, as well as improving its compatibility and moisture resistance [45]. However, the modification of alginate carboxyl groups at specific sites is critical, as multiple carboxyl groups are present in alginate.

Amidation is another chemical modification method on alginate carboxyl groups, which introduces amide groups to the carboxyl group of alginate (Fig. 4F). The introduction of amide groups can increase the water solubility of modified alginate, as well as its antibacterial performance [56]. Therefore, amidation enables the encapsulation of both hydrophilic and hydrophobic molecules [57], and provides a more biocompatible support material for enzymes [58].

Despite the advantageous properties of natural alginate for the encapsulation and delivery of enzymes, cells, and drugs, the limited stability of the encapsulation and delivery systems makes the chemical modifications of alginate an attractive approach [59]. To date, the modified alginate has been used as encapsulation materials and delivery vehicles for various biomedical applications. It would be promising to utilize modified alginate for enzyme encapsulation, in order to optimize enzyme loading capacity, release property, biodegradability, and biocompatibility. This might provide a new direction for enzyme encapsulation.

3. Fabrication methods

Numerous fabrication methods have been developed to synthesize alginate-based systems for enzyme encapsulation. Some of the prominent manufacturing techniques such as dripping and gelation, spray drying, electrospinning, fluidized bed spray coating, and microfluidics are summarized in Fig. 5, and the principles of each method are also discussed in the following sections.

3.1. Dripping and ionic gelation

Dripping and ionic gelation method is one of the most conventional techniques for producing enzyme-loaded alginate particles, due to the alginate's ability to form gels in the presence of divalent cations [63]. Basically, enzymes and alginate solution are premixed and the solution is dripped into a divalent cation gelling solution (usually Ca^{2+}) *via* a syringe (Fig. 5A). The enzyme is encapsulated inside the "egg box" structure of the alginate gel beads due to the gelation and crosslinking between alginate G-blocks and the divalent cations. The enzyme-loaded alginate beads are then soaked into the divalent cation hardening solution for several hours to complete the gelation [64], followed by a drying process.

The encapsulated enzymes synthesized from dripping and ionic gelation method showed improved stability against heat and protease, and the alginate beads properties can be controlled by varying the concentrations of alginate and cations [65]. The dripping and ionic gelation method is widely used for the preparation of enzyme-encapsulated alginate particles due to its simplicity and capability in stabilizing enzymes, however, there are also some disadvantages [7]. The dripping and ionic gelation encapsulation approach often showed a low enzyme encapsulation efficiency, due to the hydrophilicity of both alginate and most of the enzymes [66]. Additionally, enzyme leaching is

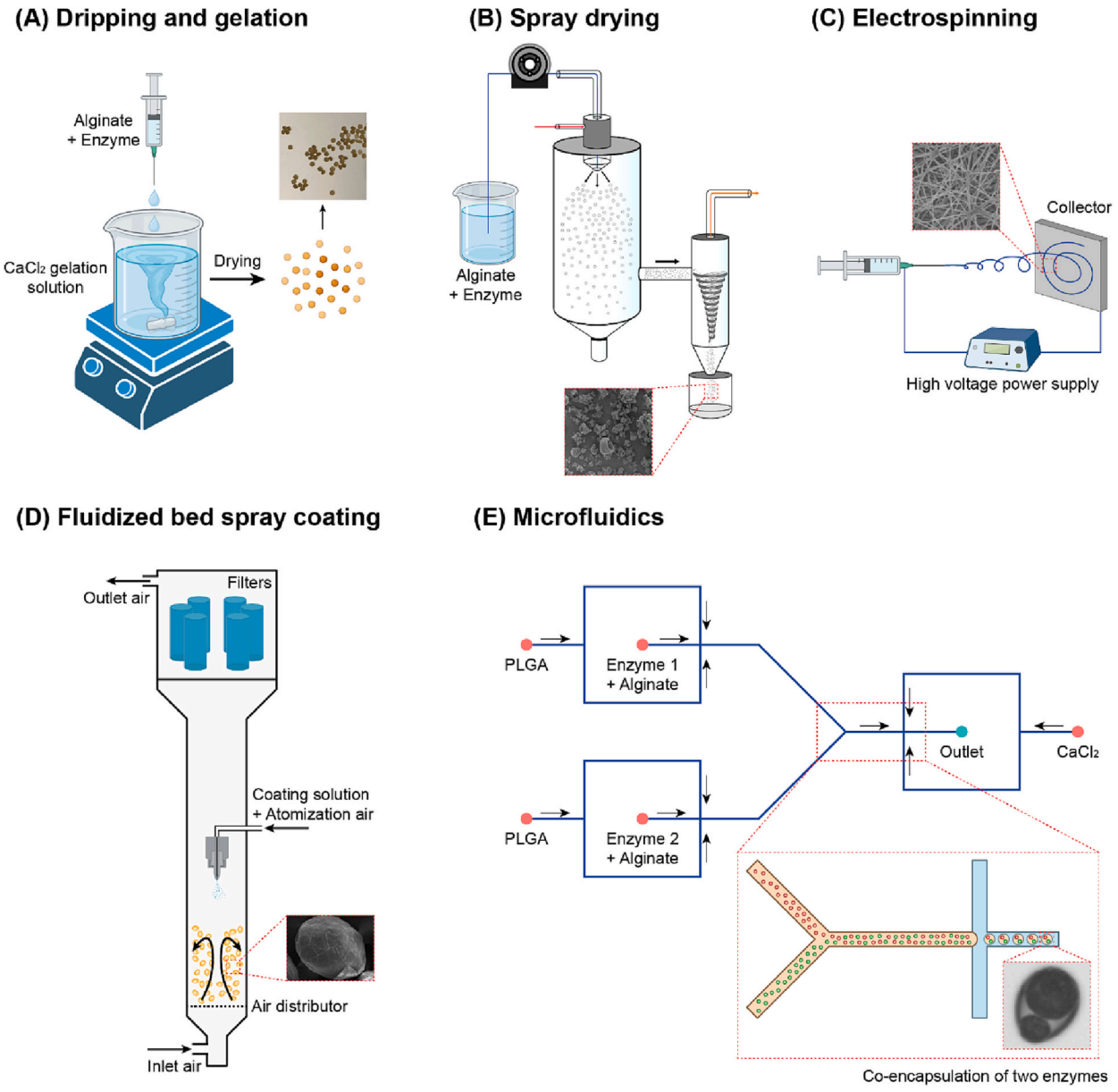

Fig. 5. A summary of fabrication techniques for alginate-based encapsulation systems (A) dripping and gelation method for fabricating alginate beads, (B) spray drying method for fabricating alginate particle powder, (C) electrospinning method for fabricating alginate fibers, SEM image from [60], (D) fluidized bed spray coating method for fabricating alginate particles, SEM image from [61], and (E) microfludics method for fabricating multi-enzyme encapsulated alginate particles, SEM image from [62].

another drawback of this fabrication method, due to the relatively large pore size of alginate hydrogel (~200 nm) [67]. Furthermore, an unsatisfactory enzyme recycling was also reported, probably due to the enzyme leaching.

The ionic gelation method can also be used to synthesize alginate films. Briefly, a solution containing a very low concentration of calcium ions is added into the film forming solution (1–3 *w*/w% sodium alginate) under magnetic stirring, followed by casting the film forming solution onto Petri dishes [68,69]. The wet alginate films are then dried overnight in oven to obtain dry alginate films.

3.2. Spray drying

Spray drying is a highly versatile drying and encapsulation technique enabling the instantaneous transformation of a liquid solution into a dry powder (Fig. 5B). Normally, a spray dryer consists of a pump, a feeding tube, an atomizer, a drying chamber, a cyclone, and a product collector, following the substance pathway from a liquid to a dry powder. The drying chamber is heated using a gas heater, and the cyclone is vacuumed. The liquid feed containing the enzyme and the coating material will undergo several steps including: (a) atomization of liquid feed, (b) droplet-particle formation, (c) particle drying, and (d) particle collection.

Spray drying manufacturing method is highly favorable in industry due to its great scalability and simple production process [70]. Alginate has been widely used in spray drying due to its gelation property. The *in situ* enzyme drying and encapsulation *via* alginate crosslinking greatly simplifies the drying process, as compared to other enzyme encapsulation techniques, enzymes often undergo two separate drying processes (after microorganism fermentation, and during the encapsulation) [71]. However, the crosslinking between alginate and divalent cations before atomization can significantly increase the solution viscosity, causing nozzle clogging. Therefore, the accurate manipulation of the gelation time is critical. To achieve this, Strobel et al. developed a pH-switching alginate crosslinking spray drying method to encapsulate bioactive food ingredients [72]. The feed stream containing the bioactive ingredient, sodium alginate, calcium carbonate, and succinic acid was spray dried after the solution pH was adjusted to 5.6 using ammonia. At this pH, the concentration of calcium ions is low, preventing the crosslinking before atomization. During atomization, with the evaporation of ammonia, the pH of the feed solution drops, enabling the crosslinking of calcium and alginate during the spray drying process [73].

Although encapsulating enzymes using alginate spray drying can significantly improve enzyme stability, the alginate shell might hamper enzyme activity. Estevinho et al. encapsulated beta-galactosidase in alginate polymers using spray drying, and the stability of the microparticles was improved as suggested by the large zeta potential (below −40 mV) [74]. However, only 20% of enzyme activity was retained after microencapsulation, probably due to the microparticle porosity and shell resistance, which limits the diffusion of both enzyme and substrate to and from the microparticle. To mitigate the effect of alginate shell resistance on enzyme activity, an addition of another polymer as the encapsulation material is an effective approach. For example, in another spray drying encapsulation study, Lakshmi et al. added polyethylene glycol (PEG) to the spray drying feed, which improved the lipoxygenase activity recovery rate from 50% to 72%. Meanwhile, the enzyme thermal stability and storage stability were both improved [75]. This remarkable enzyme activity recovery rate improvement might be due to the alteration of the enzyme hydration shell, which adjusted the shell resistance and enhanced enzyme conformational stability.

Additionally, limited enzyme loading capacity is another common drawback for spray drying enzyme encapsulation. There is a tradeoff between enzyme loading capacity and enzyme stability, as a higher enzyme loading capacity can reduce the cost of the coating material, while it might compromise the stability of encapsulated enzymes. To optimize enzyme loading capacity and stability in the spray drying encapsulation process, Weng et al. employed a novel three-fluid spray drying nozzle for the microencapsulation of phytase, and the enzyme loading capacity was improved from 20% to 48%, compared to their previous study using a conventional two-fluid spray drying nozzle, and enzyme thermal stability was increased almost four folds [6].

To maximize enzyme activity after spray drying, it is critical to control the spray drying operational parameters such as air heating temperature and feed rate for enzyme encapsulation works. For example, a high heating temperature (*e.g.* above 200 °C) can easily deactivate enzymes, and the product moisture content might be high if the feed rate is too fast, which can negatively impact the enzyme storage stability.

3.3. Electrospinning/electrospraying

Electrospinning is a broadly used electrostatic fiber formation technology which uses electrical forces to synthesize polymer fibers [76]. The system simply consists of a power supply, a delivery system (usually a syringe pump), and a grounded collector (Fig. 5C). With the introduction of a strong electric field (typically several kV), the polymer melts and forms a fibrous mat with ten to hundred micrometers in diameter (Fig. 5C). Electrospinning has been applied for enzyme encapsulation due to its ability to generate fibers and particles with high surface area, versatile architectures, room temperature synthesis, and highly controllable release profiles [77]. Although conventional hydrophobic polymers such as poly (lactic-*co*-glycolic acid) (PLGA), polycaprolactone (PCL), and polylactic acid (PLA) are mainly used for enzyme encapsulation *via* electrospinning [78,79], classical hydrophilic polymers like alginate are also utilized. Water-in-oil emulsions are used for encapsulating hydrophilic molecules such as enzymes [77]. Briefly, an enzyme is dissolved in an alginate aqueous phase, and then mixed with an organic solvent to form water/oil emulsions. Upon the evaporation of the organic solvent and water phases, alginate particles containing enzymes are formed [80].

Enzyme encapsulation using electrospinning is effective for improving enzyme stability, especially suitable for thermal sensitive enzymes. Zdarta et al. stabilized horseradish peroxidase (HRP) in alginate-poly(vinyl chloride) (PVC) electrospun fibers with an 80% activity recovery rate, and the enzyme storage stability was improved to 60% after 20 days, while free enzyme only remained 20% of the initial activity [81]. In another study, glucose oxidase (GOx) was encapsulated in alginate capsules using both dripping and electrospraying method [82]. Both methods improved enzyme stability, however, enzyme encapsulated *via* dripping method showed an even higher stability against pH and thermal stresses, as reported.

Despite the current success in using electrospinning as an enzyme encapsulation technique, the low production yield of electrospinning and the questionable scalability are the major drawbacks. Therefore, future studies are still required to improve the production yield as well as scale up the current lab-scale production techniques into an industrial scale. Additionally, there are lots of opportunities for applying new polymers as copolymers, blends or composites in electrospinning for enzyme encapsulation, and various perturbations of electrospinning such as core–shell electrospinning can be employed [83].

3.4. Fluidized bed spray coating

Fluidized bed spray coating is a conventional process for particle coating in various industries due to its large-scale manufacturing capability (Fig. 5D) [84]. Fluidized bed coating was first reported as an enzyme immobilization method by Gerard in 1974, when a magnetic support was used to crosslink the enzyme with an inactive protein, to improve the reusability of the enzyme [85].

The fluidized bed coating and the spray drying method shared lots of common properties as they have both demonstrated a high scalability and are capable for *in situ* enzyme coating and drying for enzyme

encapsulation. Nevertheless, the sizes of the fluidized bed coated products (around 200 μm) are much larger than the sizes of the spray dried encapsulated particles (around 10 μm) [86]. This major difference makes the enzyme release from fluidized bed coated particles much slower than the enzyme release from the spray dried encapsulated particles, due to the limited surface area. Another major difference is the particle retention time (60–300 min for fluidized bed coating *versus* several seconds for spray drying) [87]. Enzyme stabilizers such as trehalose or sucrose might be required for the fluidized bed coating method to avoid the loss of enzyme activity during this long coating period. Therefore, the selection of a fabrication method for enzyme encapsulation should be made with careful consideration based on the specific application requirements.

Although the fluidized bed spray coating technique has been applied to manufacture coated enzymes in many commercial products, it faces challenges such as particle agglomeration, attrition, and low coating efficiency, and a poor production yield [88]. Kawakita et al. developed a fluidized bed enzyme spray coating process for crosslinked alginate, and the encapsulated particles showed improved mechanical strength [89]. The *in situ* crosslinking of alginate and $CaHPO_4$ resulted in an improved spray coating rate, and a high process yield, mainly due to the consistent inhibition of the formation of fines which would otherwise cause excessive filter fouling. Although the viscosity of the feed suspension was raised due to the incorporation of alginate, the size of the spray droplet was still kept small by increasing nozzle pressure, which avoids overwetting [89].

3.5. Microfluidics

Microfluidic fabrication technique is an emerging approach to manufacturing customizable structures in a small scale (Fig. 5E) [90,91]. Alginate-based materials such as alginate microfibers and microparticles can be fabricated using microfluidic methods, with great flexibility on controlling the size, composition, and pore size of the materials [39,92]. For example, one of the common drawbacks of crosslinked alginate as enzyme encapsulation material is enzyme leakage, due to the porous alginate hydrogel structure. Microfluidic fabrication enables the manipulation of alginate pore size by tuning the degree of crosslinking. Recently, Zhang et al. encapsulated glucose oxidase (GOx) and horseradish peroxidase (HRP) in alginate microfiber using microfluidic fabrication technique, and over 85% of enzyme activity was retained after seven cycles [39]. In another recent study, the compartmentalized encapsulation of both glucose oxidase (GOx) and catalase (CAT) was realized using microfluidic technology [62]. The encapsulation improved the enzyme stability, especially under alkaline intestinal environment, and the size of the fabricated particles can be controlled by simply tuning the flow rates. More importantly, enzyme-catalyzed reactions can be achieved due to the pH-responsive and biocompatible nature of alginate. Furthermore, the microfluidic fabrication method allows the controlled release of encapsulated substances by an addition of polymers. Yu et al. successfully tuned the release profiles of ovalbumin (OVA) and engineered the alginate microparticle structures by adding poly(ethyleneimine) (PEI) and chitosan [93]. The protein release was prolonged upon the PEI coating on the alginate microparticles compared to those in the absence of PEI coating.

Nevertheless, it should be noted that most studies used microfluidic fabrication methods for the encapsulation of cells and other hydrophobic materials [94,95]. The good water solubility of most enzymes greatly limits the encapsulation efficiency, as both enzymes and alginate are dissolved in the water phase, where enzyme leakage is almost inevitable. It is challenging to address the limited enzyme encapsulation efficiency for both the microfluidic fabrication method and the dripping and ionic gelation method. Overall, due to the tunability and versatility, the alginate-based microfluidic manufacturing platform may provide a valuable approach for enzyme encapsulation in an environmentally friendly and biocompatible way.

4. Characterization of enzyme-alginate composites

To characterize the physical and chemical properties of enzyme-loaded alginate composites, a variety of analytical techniques can be used. To visualize the shape, size, and surface morphology of alginate particles, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are most commonly used. Besides, dynamic light scattering (DLS) is a promising technique for the analysis of particle size, size distributions, and surface charges, however, samples incompatible with water solutions cannot be used for DLS characterization. To study the distribution of both alginate coating material and enzymes, fluorescence dyes such as fluorescein isothiocyanate (FITC), cyanine 3 (cy 3), cyanine 5 (cy 5), and rhodamine B can be used to label the polymer and the enzymes, and confocal laser scanning microscopy (CLSM) is often used to observe their distributions. The alginate structure at molecular level is mainly characterized using Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), X-ray photoelectron spectroscopy (XPS), X-ray powder diffraction (XRD), and Raman spectroscopy (RS). Small-angle X-ray scattering (SAXS) is another precise and powerful technology to evaluate hydrogel microstructures, which is able to reveal subtle differences in electron density within hydrogel network ranging from 1 to 100 nm [28]. Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) are the most widely used thermal analysis techniques for the evaluation of enzyme thermal stability. Circular dichroism (CD) is another characterization technique to evaluate the conformational stability of enzyme structure. For enzyme quantification and purification, ultraviolet-visible spectroscopy (UV–Vis), high-performance liquid chromatography (HPLC), and ÄKTA chromatography (AKTA) purification are often used. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and liquid chromatography–mass spectrometry (LC-MS) are reliable methods to measure molecular mass of enzymes.

5. Release of encapsulated enzymes from alginate materials

5.1. Release mechanisms

Encapsulation of enzymes within alginate materials has been a topic of great interest in various fields such as food science and pharmaceuticals. Enzyme bioavailability is of great importance for the potential applications of encapsulated enzymes, especially in the food and feed industry where enzymes are frequently used as food/feed additives [96]. The extent to which the enzymatic activity can be released and utilized is important, however, lots of studies have reported a compromised bioavailability of encapsulated enzymes [6]. Both *in vitro* release studies and *in vivo* animal trials can be used to investigate the bioavailability of encapsulated enzymes. *In vitro* release studies can simulate the gastrointestinal conditions of humans/animals including the pH, temperature, and proteases, which are much cheaper, easier, and quicker to operate than conducting *in vivo* animal trials. Understanding the release mechanisms of various enzyme release studies can help researchers optimize the design of the controlled enzyme release systems.

The release of the encapsulated enzymes from alginate materials can be accomplished through various mechanisms, which are based on the degradation of the alginate matrix. Three main mechanisms of enzyme release from alginate matrix include swelling, erosion, and diffusion (Fig. 6) [97]. Swelling is a process where the uptake of water into the alginate matrix results in its expansion, leading to the release of the encapsulated enzymes [98]. Erosion, another physical mechanism, takes place when the alginate matrix gradually wears away, releasing the enzymes over time. Diffusion is yet another major release mechanism, where the enzymes diffuse through the alginate matrix in a random movement, which is guided by a potential chemical gradient [99]. Diffusion is also governed by the solubility and permeability of the enzymes. Diffusion and swelling are the dominant mechanisms for

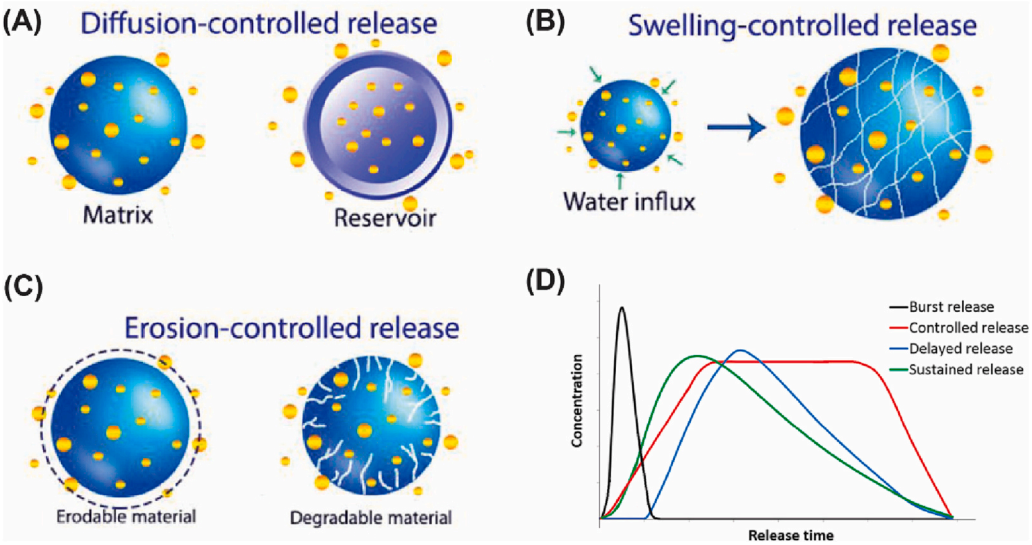

Fig. 6. A schematic illustration of diffusion (A), swelling (B), and erosion (C) controlled release systems, adapted from [102] with permission, and common release profiles for encapsulated enzymes (D), adapted from [100] with permission.

alginate-based encapsulation systems [100]. Due to the pH responsive property, alginate is often used for the design of pH triggered enzyme release, which have found numerous applications in food and pharmaceutical industries [101]. Overall, the release mechanisms of encapsulated enzymes from alginate materials are crucial in determining the success of their applications. Further research is needed to fully understand the underlying mechanisms and to optimize the release of enzymes from alginate materials.

5.2. Release profiles and mathematical release models

The release profile of an encapsulated enzyme shows the change of enzyme concentration over time, which is mainly affected by the nature of release mechanisms [103]. Common release profiles are burst release, triggered (controlled) release, sustained release, and delayed release, and relevant patterns of these release profiles are summarized in Fig. 6D. To compare these different release profiles, and to predict the effect of process parameters and formulations on release kinetics accurately and quantitatively, mathematical release models are proposed [102]. Some of the most widely used release models are zero-order model, first-order model, Higuchi model, Hixson-Crowell model, and Korsmeyer-Peppas model (Table 1).

Burst release is defined as the initial rapid release of enzymes, which is generally uncontrolled. Alginate-based encapsulation systems are normally designed to achieve sustained release, as well as triggered release. A triggered release can be employed when the rapid release of the encapsulated enzymes at certain spots is desired. Triggered release is often designed for food related applications, where bioactive ingredients such as enzymes [109], ascorbic acid [110], and tea polyphenols [111] are encapsulated in alginate particles to improve stability and released promptly at targeted sites as desired [112]. Weng et al. achieved a rapid release of phytase from spray dried alginate particles at targeted sites and over 98% of enzyme was successfully released after 105 min, at the simulated chicken gastrointestinal tract (GIT) conditions [6]. It was observed that with the decrease of pH from 5.5 to 3.5, the release rate was significantly reduced, due to the impeded alginate swelling at low pH environments. First order model best fits the release profile and the enzyme release was dominated by diffusion [6]. Both the structure porosity and the distribution of enzymes within alginate coating materials contribute to the rapid enzyme release at the targeted sites. In another example, Wang et al. observed a rapid release of peptide (E7 and B2A) from porous alginate-polycaprolactone (PCL) structure, and the release rate was reduced when the porosity of the material went down [113]. It has also been reported that the presence of a porous structure increased the drug release while non-porous hydrogels led to a very slow release [114].

In comparison with the rapid release profiles, sustained release is defined as a slow and long acting release over a period of time [100]. Santagapita et al. achieved a sustained release of invertase from alginate

Table 1
A summary of common release models and their applications.

model	equation	parameters	applications	reference
zero-order	$M_t = M_0 - kt$	M_0: the initial concentration of the encapsulated substance M_t: the concentration at time t M_∞: total amount of substance encapsulated k: release constant, dependent on the structural and geometrical characteristics of the release system n: release exponent, dependent on the release mechanism	The release of enzymes at a constant rate	[104]
first-order	$\frac{M_t}{M_\infty} = 1 - e^{kt}$		This model best describes the release kinetics of hydrophobic materials in porous matrixes.	[105]
Higuchi	$\frac{M_t}{M_\infty} = kt^{1/2}$		This model best describes the release kinetics of hydrophobic materials from insoluble matrixes.	[106]
Hixson-Crowell	$M_0^{1/3} - M_t^{1/3} = kt$		This model best describes the release kinetics of materials from planar geometries where dissolution occurs in parallel with the surface area of the matrixes.	[107]
Korsmeyer-Peppas	$\frac{M_t}{M_\infty} = kt^n$		This model is often used when more than one release mechanism is involved. It is only used when the amount of material released is below 60%.	[108]

beads and the release rate was controlled by changing the beads drying method [115]. According to their work, enzyme release from vacuum-dried alginate beads was much quicker than enzyme release from freeze-dried alginate beads due to the pore size differences. In another study, Yan et al. encapsulated alfacalcidol in cellulose-alginate composite beads, and a sustained release was achieved by the external gelation of hydrogel shells and the interfacial assembly of amphiphilic beads, resulting in a low cytotoxicity, which promotes osteoblast differentiation [116]. Therefore, various enzyme release systems can be designed based on the demand, by controlling the precursor concentrations, drying method, material porosity, enzyme distributions, presence of extra coating material, and degree of crosslinking of the alginate-based material.

6. Applications of alginate in enzyme encapsulation

Alginate-based enzyme encapsulation systems have found various industrial applications, mainly in the areas of food and beverage, agricultural, environmental remediation, and pharmaceutical industries, which are discussed in the upcoming sub-sections. Other applications such as detergent and biocathode making, toothpaste formulation, design of new catalytic system, and fertilizer development are also highlighted. Table 2 summarizes the applications of encapsulated enzymes in alginate-based materials for various industrial applications, as well as their key findings and preparation methods.

6.1. Food and beverage industry

Enzymes have been widely used in food and beverage industry, such as food and beverage manufacturing, quality control and flavor development, food additives, and food preservation. For examples, glucose oxidase can catalyze the oxidation of glucose, enabling a longer shelf-life for food, as well as improves flavors [137]. Catalase is used in food packaging and preservation processes, catalyzing the decomposition of hydrogen peroxide [138]. Laccase and alpha-amylase are widely used in baking, brewing and starch liquefaction [111]. Lactase is of great importance for lactose-free milk production, as lactose intolerance is common for many people [139]. Lipase is essential for fat and oil processing, as well as flavor development for dairy products [140]. Proteases and peptidases are also utilized in baking industry for the reduction of overall food preparation time, regulating the bread gluten strength as well as improving its flavor [141]. Phytase is used as feed additives in feed industry to promote the digestion and adsorption of phosphorus [142].

Despite these great functionalities of enzymes, their poor storage stability and high sensitivity to unfavored conditions such as heat, pH, and organic solvents greatly limit their practical applications in food and beverage industry. When exposed to such undesired environmental conditions, their activities are often compromised, or even completely lost [12,143]. Encapsulating enzymes in alginate-based materials is a useful approach to improving enzyme stability and extending its shelf life. Conventionally, the crosslinked alginate gel beads provide a significant protection for enzymes against elevated temperatures and changing pHs.

Recently, the incorporation of alginate with other organic/ inorganic materials as encapsulation supports has attracted great interest. Tavernini et al. employed alginate-PVA in glycosidase encapsulation and the enzymatic activity was fully retained after 140 incubation days [121]. Similarly, Piacentini et al. have improved the operational stability of lipase, promoted the intrinsic optimal interaction between the enzyme and substrate using alginate-PVA encapsulation [144]. Alginate-chitosan composite material is another popular candidate for encapsulating enzymes in food related applications, due to their non-toxicity and biocompatibility. The interactions between the positively charged chitosan and the negatively charged alginate can provide a smoother capsule, which is less permeable to heat and hydrophilic substances, further protecting the enzymes [145]. Lysozyme [119], naringinase [122], and rennet [61] have been encapsulated in alginate-chitosan composite materials for food preservation, juice debittering, and cheese making, respectively, with remarkably increased thermal and pH stability.

6.2. Pharmaceutical industry

Enzymes are essential in the synthesis of various drugs and other pharmaceutical compounds through various reactions such as selective acylation and deacylation, selective hydrolysis, deracemization, esterification, and transesterification [146]. Besides, enzymes themselves can be used therapeutically as drugs due to their economic viability and reliability [147]. Immobilized enzymes are also used in pharmaceutical industries for a higher enzyme stability [148]. Ward et al. used sodium alginate for the first time as a potential ligand for enhanced colloidal liquid aphrons (CLA) immobilization, using lysozyme as a model enzyme [124]. The CD results confirmed the unchanged protein secondary structure after encapsulation. The enzyme activity retention makes alginate-CLAs a strong candidate for enzyme immobilization and drug delivery in pharmaceutical industry. In another study, Buamard et al. encapsulated trypsin in alginate beads with a higher stability against structural breakdown in the simulated stomach environment [123]. The encapsulated trypsin might serve as digestion aid supplements which enhances proteolysis within the intestinal tract. Additionally, the pH responsive nature of the alginate-based materials can achieve the target delivery and controlled release of therapeutic enzymes at small intestines as the pH increases throughout the gastrointestinal tract.

6.3. Biosensing

Biosensors are devices that detect biological substances or changes in biological activity [149]. Alginate encapsulated enzymes are a promising development in the field of biosensors and have generated considerable interest in recent years. One of the major advantages of alginate encapsulated enzymes is their high stability and long-term storage potential, which makes them an attractive option for use in biosensors. They can also be easily immobilized on a solid support, such as a glass electrode, which enhances their sensitivity and accuracy in detecting target analytes [150].

Several studies have demonstrated the effectiveness of alginate encapsulated enzymes as biosensors. For example, Zhao et al. used an alginate-encapsulated glucose oxidase to detect glucose levels in human serum samples with high accuracy and sensitivity [151]. Similarly, Buk et al. developed an alginate-CuO-glucose oxidase based biosensor for the detection of glucose, showing both good reproducibility and long-term stability [152]. In another study, horseradish peroxidase was encapsulated in calcium alginate beads for the detection of hydrogen peroxide in environmental water samples [153]. Overall, alginate-based biosensors have great potential for biomedical applications due to the simplicity, low cost, and high sensitivity and selectivity.

6.4. Wastewater treatment

Contamination of water bodies with different classes of artificial pollutants including suspend solids, heavy metals, nutrients (nitrogen and phosphorus), pathogens, and pesticides can have devastating effects on human and aquatic lives. Enzymes, especially laccase and peroxidase enzymes, are utilized for wastewater treatment due to their ability to degrade different classes of organic compounds [154]. Alginate-based materials are widely used as support materials to improve the operational stability of these enzymes and promote their adoption as a cost-effective and recyclable remediation approach.

Horseradish peroxidase (HRP) is an enzyme commonly used for the removal of reactive dyes in wastewater through biodegradation,

Table 2
A summary of alginate encapsulated enzymes studies: major findings, industrial applications, and preparation methods.

enzyme	alginate material	preparation method	EE	loading	major findings	applications	reference
bromelain	sodium alginate	complex coacervation		19.50%	the process maintained maximum enzyme activity (~100%) and facilitated the incorporation of enzymes into food products in a cost-effective way	food industry	[117]
rennet	alginate-chitosan nanoparticle	ionotropic gelation and polyelectrolyte complexation	~45%		improved pH stability (pH 3–9), improved thermal stability at 50–70 °C, fastest release observed under acidic conditions (pH 4, 20% released in 8 h)	food industry	[61]
amylase	Ca alginate	cross-linked enzyme aggregates			improved thermal stability, reusability, organic solvent resistance, reduced enzyme leakage.	food industry	[118]
lysozyme	alginate-chitosan hydrogel	dripping			prolonged antibacterial activities, 87% of enzyme activity released	food preservation	[119]
glucose oxidase (GOx)	mucilage-sodium alginate	electrospraying and dripping			both methods had improved enzyme pH and thermal stability, and the dripping method showed an even better enzyme stability	bread making	[82]
α-acetolactate decarboxylase	$Ca_3(PO4)_2$-alginate nanoflower	co-precipitation, dripping			improved recyclability and pH stability at basic pHs	beer brewing	[120]
glycosidases	alginate-PVA	cross-linked enzyme aggregates		60 mg/g support	improved operational stability, retained full activity after 140 incubation days	wine aroma enhancement	[121]
naringinase	alginate-chitosan nanocapsule	dripping			improved pH and thermal stability	juice debittering	[122]
trypsin	alginate-chitosan beads	alginate beads (dripping)	80%		improved stability under stomach environment, which enhanced protein digestion	drug delivery	[123]
lysozyme	alginate-colloidal liquid aphron (CLA)	CLA-alginate reaction			the encapsulation was optimized when the bulk pH was below PI, due to the strong interaction between two oppositely charged molecules. CD results confirmed the unchanged protein secondary structure after encapsulation (only electrostatic interaction occurred)	enzyme immobilization and drug delivery	[124]
pyruvate ferredoxin oxidoreductase	Ca alginate	dripping			improved thermal and operational stability, over 68% initial activity retained after 10 repeated cycles.	acetyl-CoA production, pharmaceuticals manufacturing	[125]
tyrosinase (TYR) and β-glucosidase (β-Glu)	magnetic alginate-polydopamine (PDA) beads	magnetic alginate-PDA gelation			improved pH stability at basic pHs, optimal temperature changed from 25 to 35 °C, improved storage stability up to day 35	pharmaceutics, biocatalysis, and biosensing	[126]
urease	alginate nanogel	water-in-oil emulsion	70%	0.07%	improved thermal stability	drug delivery, biosensing	[127]
urease	Ca alginate nanogel	microemulsion polymerization	64%	6.40%	improved storage stability up to 30 days	urea detection	[128]
glucose oxidase (GOx), horseradish peroxidase (HRP)	alginate microfiber	microfluidic fabrication	95%	66%	improved thermal stability at 60 °C, improved recyclability	cascade catalysis and diagnoses with multiple clinical markers	[39]
horseradish peroxidase (HRP)	alginate-poly(vinyl chloride)	electrospinning		25 μg/1 mg support	improved storage stability and recyclability	wastewater treatment	[81]
glucose oxidase (GOx), catalase (CAT)	alginate-PLGA	microfluidic fabrication			pH triggered enzyme-catalyzed reactions were achieved using pH-responsive alginate cores	biocompatible fuel, co-encapsulation, and autonomous movement in multiple applications	[62]
laccase	Cu alginate	crosslinking with electropolymerized polyaniline			improved recyclability, remained high dye decolorization efficiency (81%)	biocathode making	[129]
subtilisin protease	cross-linked alginate matrix shell	fluidized bed spray-coating			encapsulated particles showed improved mechanical strength and minimal surface damage	bioactive cargo protection	[89]
tyrosinase	Cu alginate gel	alginate beads (dripping)			improved thermal stability and pH stability (pH 5.5–7)	catecholic production with high recyclability and productivity	[130]
dextranase	Ca alginate	alginate beads (dripping)	8–80%		strong inhibition of bacteria growth, improved storage	toothpaste formulation	[131]

(*continued on next page*)

Table 2 (*continued*)

enzyme	alginate material	preparation method	EE	loading	major findings	applications	reference
					stability, optimal pH and temperature were changed to pH 6 and 37 °C for dental applications		
β-glucosidase	alginate-silica hybrid hydrogel	internal gelation			improved operational stability.	design of new catalytic system	[132]
acetylcholinesterase	Ca-Mg-alginate	alginate hydrogelation			improved storage stability up to 5 days	capillary electrophoresis	[133]
esterase	alginate-chitosan beads	dripping	88%		improved storage stability. Enzyme activity as well as encapsulation efficiency were notably enhanced by chitosan coating, enzyme leaching reduced by bead acidification	acid production	[26]
extracellular alkaline protease	alginate, white clay, and kaolin	alginate beads (dripping)	35.50%		improved alkaline pH and temperature stability	laundry detergent	[134]
urease	Ca alginate	gelation			improved thermal stability (60–80 °C), improved storage stability up to 30 days	fertilizer development	[135]
glucose oxidase (GOx)	graphene oxide-alginate beads	dripping			improved mechanical strength and enzyme stability, reduced enzyme leakage, enzyme optimum activity retained within a broad range (temperature 45–60 °C, pH 4–6)	continuous enzymatic reaction	[136]

however, the decolorization efficiency is greatly limited by the poor enzyme stability and recyclability. Encapsulating HRP in alginate beads is an effective strategy to enhance its stability, and a 20% increase of enzyme activity after seven cycles was reported by Janovic et al. [155]. In another study, Wei et al. reported a remarkably enhanced operational stability and recyclability of mushroom tyrosinase for the elimination of phenolic pollutants in wastewater, after the encapsulation in alginate-PVA materials [156].

7. Future perspectives

As we look to the future, the alginate-based materials for enzyme encapsulation and the production processes are likely to evolve considerably. Further optimization of the encapsulation process is still necessary to fully exploit the potential of alginate. Improving our current understanding of the fundamental properties of alginate and alginate-based materials, as well as developing new encapsulation systems, such as single enzyme encapsulation, nanoencapsulation, and multi-enzymes encapsulation systems may enable future advances in material science and engineering. Additionally, designing new classes of alginates with precisely controlled chemical and physical properties for specific applications can revolutionize the function and application of alginate-based materials. Furthermore, more process scalability studies are required to convert lab-scale researches into industrial applications, as most of the current enzyme encapsulation studies are still limited in lab-scales.

7.1. Single enzyme encapsulation

Although the encapsulation of enzymes in matrices is a well-established technique, the precise control of the individual enzymes is an emerging field. Single enzyme encapsulation (SEE) has emerged as a novel enzyme encapsulation technique which has several advantages over the conventional encapsulation techniques, including the ability of controlling both enzyme stability and enzyme activity [157]. SEE has shown significant enzyme stability improvement as well as high enzyme activity recovery for quite fragile enzymes, due to the increased control of both substrate diffusion and the environment directly around the enzyme [158]. For SEE, substrate diffusion is only governed by the property of the encapsulating layer, instead of the contact and interaction between enzymes, therefore, the enzyme activity as well as the substrate diffusion can be tuned more easily by simply adjusting the thickness and porosity of the encapsulating layer. The emerging SEE technique might also enable enzyme activity to be switched on and off on demand by modulating the encapsulating layer, as well as extend the enzyme catalytic lifetime at extreme temperatures and aggressive solvents. Overall, single enzyme encapsulation is an emerging promising technique that offers ample opportunities for the customization of enzymes towards individual applications, and the potential of alginate as being an encapsulating material for SEE could be explored.

7.2. Nanoencapsulation

Nanoencapsulation is another emerging technique which particularly improves the bioavailability and sensitivity of the encapsulated substances. Compared with conventional encapsulation methods, nanoencapsulated enzymes present a much higher surface-volume ratio, increasing the contact area between the enzyme and substrate, which promotes enzyme bioavailability and sensitivity. Although enzyme nanoencapsulation has been achieved *via* alginate nano-spray drying [159], the efficient nanoparticle collection remains a challenge. Besides, the slow evolution of security and regulations of nanoparticles has limited the applications of enzyme nanoencapsulation [160]. Furthermore, further studies on health and safety are required as the long-term impact of these nanoparticles on both human health and the environment is still unclear.

7.3. Multi-enzymes encapsulation systems

Although alginate-based encapsulation systems for encapsulating single types of enzymes haven been extensively studied, the development of multi-enzymes encapsulation systems is emerging as a promising trend. According to the pioneer research, the co-encapsulation of multiple bioactive ingredients offers lots of benefits, such as improved activity, stability, bioavailability, and physiological functions [62,161]. Despite these advantages, limitations still remain for the co-encapsulation systems, such as the low encapsulation efficiency and the unclear mechanism [16]. As there are only few studies focusing on alginate-based multi-enzymes encapsulation systems, huge potential might be explored in further studies.

8. Conclusions

In conclusion, alginate-based materials have demonstrated great potential for enzyme encapsulation in various industrial applications, particularly in areas of food and beverage, agricultural, environmental remediation, and pharmaceutical industries. For these applications, the most attractive features of alginate include biocompatibility, ease of use, simple preparation of alginate derivatives with tunable properties *via* chemical modifications, and mild gelation conditions. The encapsulated enzymes present significantly improved stability under unfavorable conditions, compared with native enzymes. This review provides an extensive investigation of different alginate-based enzyme encapsulation systems, such as alginate spheres (gel beads and emulsions), alginate films, and alginate fibers. Different enzyme release systems can be designed based on the individual application to maximize enzyme bioavailability and efficiency. Furthermore, various fabrication methods for enzyme encapsulation are discussed, including dripping and gelation, spray drying, electrospinning, fluidized bed spray coating, and microfluidics. Further investigations on the development of new classes of alginate-based materials, process scale-ups, single enzyme encapsulation, nanoencapsulation, and multi-enzymes encapsulation could contribute to the future advancement of alginate-based encapsulation systems.

CRediT authorship contribution statement

Yilun Weng: Writing – original draft, Formal analysis, Writing – review & editing. **Guangze Yang:** Writing - review & editing. **Yang Li:** Writing - review & editing. **Letao Xu:** Writing - review & editing. **Xiaojing Chen:** Supervision. **Hao Song:** Supervision, Writing - original draft. **Chun-Xia Zhao:** Supervision, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

All authors declare no competing interest.

Data availability

No data was used for the research described in the article.

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