Enzyme Immobilization for Commercialization in Textile Processing

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***Abstract*—Enzymes are biological molecules constituting proteins derived mainly from living organisms. Owing to their diverse applications, enzymes have become indispensable across many industries significantly streamlining complex manufacturing processes. Enzymatic processes have become more prominent in the textile industry over the past decades due to their environmentally friendly benefits. Enzymes have several applications in the textile industry, ranging from pre-treatment of fabric to finishing and from decolorization to effluent treatment. However, the conventional application of enzymes in pure form presents certain challenges, such as loss of enzyme and limitations in enzyme recovery. These challenges can be addressed by means of immobilization. It saves enzyme wastage, makes the enzyme reusable. This review summarizes the research on enzyme immobilization techniques, supporting materials and methods used for immobilization. It further throws light on why immobilization is not commercialized at industrial scale in the textile industry.**

***Keywords*— Enzyme-assay, Cross-linkers, Micro-encapsulation, Effluent-leaching, Sustainable processing**

I. INTRODUCTION

Enzymes act as catalysts, accelerating specific reactions and have diverse industrial applications based on their catalytic abilities. Enzyme immobilisation is a process of attaching an enzyme to a solid supporting material [1]. This process requires the presence of an enzyme, a matrix and a mode of attachment [2]. There are multiple modes and mechanisms reported in literature for attaching enzyme in a matrix. Immobilized enzyme supports enzyme survival and also minimizes enzyme wastage. This helps to speed up the reaction more prominently as compared to enzymes in free form[3]. The various modes and mechanisms of attaching enzymes in a suitable matrix for immobilization are explained below in detail.

II. MECHANISMS OF ENZYME IMMOBILIZATION

Enzyme immobilization can be done by various mechanisms like adsorption, covalent bonding, sol-gel method, ionic bonding, entrapment, cross-linking and encapsulation. The adsorption process attaches the enzyme to the surface of the supporting material. Recently, researchers have reported enzyme immobilization on a biosensor transducer by the adsorption method[4]. Immobilization done via covalent bonding forms strong, stable bonds between the enzyme functional group and chemically activated support[5]. Whereas the sol-gel method of immobilization involves two phases. A sol is a colloid with solid particles dispersed in a fluid medium, and the gel is a colloidal network expanded by a fluid forming a semi-solid structure[6].

Sol-gel method enables low-temperature, biocompatible encapsulation of heat-sensitive enzymes[7]. Ionic bonding attaches enzymes to a charged support via electrostatic interactions[8]. Entrapment method ensures the enzyme is entrapped within the supporting material[9]. By means of cross-linking, intermolecular bonds and cross-linked networks are formed between enzymes and cross-linking agents like glutaraldehyde, which result in insoluble and stable aggregates[10]. Other methods, like encapsulation enclose enzymes in semipermeable membranes like vesicles or microcapsules that shield the enzymes[11]. Figure 1 summarizes the modes and mechanisms of enzyme immobilisation. These mechanisms of immobilizing enzymes are discussed in detail in the subsequent sections.

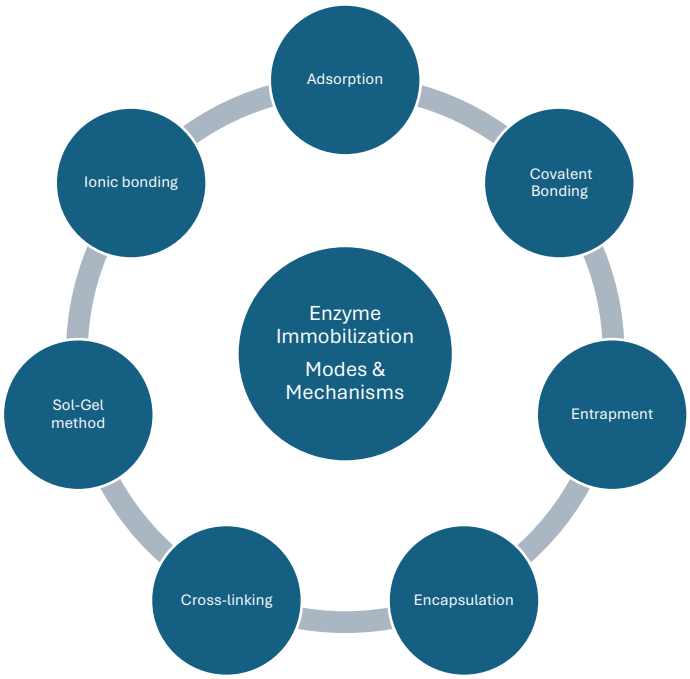

Figure 1 - Modes and mechanisms of enzyme immobilization

III. SIGNIFICANCE OF ENZYME IMMOBILIZATION IN TEXTILE INDUSTRY

Textile industries use harsh chemicals in their processing stages. Enzymatic processes offer eco-friendly wet processing pathways, which are safer and more environmentally friendly. Immobilization serves longer enzyme life, better catalysis for textiles, which aligns with sustainable goals. Enzymatic immobilisation reduces effluent treatment costs and improves efficiency. Immobilization provides distinct benefits compared to the conventional use of free enzymes in the textile wet processes. Free enzymes are typically single-use and are easily deactivated by extreme pH conditions, high temperatures, mechanical stress and prolonged processing times[12].

In contrast, immobilised enzymes demonstrate improved thermal stability. Unlike free enzymes that are lost with the processing bath after each cycle, immobilized enzymes can be recovered and reused over multiple batches or in continuous operations. This can lead to a significant reduction in enzyme consumption and processing costs. Immobilization also allows greater control over enzymatic activity, which ultimately eliminates issues such as over-processing and excessive fibre weight loss. Furthermore, immobilization reduces the discharge of enzymes and biological molecules into effluents, thus lowering wastewater load and environmental impact. Overall, enzyme immobilization addresses the limitations of free enzyme systems by enhancing process efficiency, consistency, sustainability and cost-effectiveness in the textile industry. Table1 summarises the prominent categories of enzymes and their applications in the textile industry [13].

Table 1- Enzymes used in Textile processing

Enzyme class	Enzyme	Textile application
Oxidoreductases	Laccase (EC 1.10.3.2)	Textile effluent decolorization, denim bleaching, oxidative degradation of dyes and phenolic compounds
	Peroxidase (EC 1.11.1.7)	Oxidative degradation of phenolic and aromatic dye compounds (including azo dyes in the presence of mediators)
	Catalase (EC 1.11.1.6)	Decomposition of residual hydrogen peroxide after bleaching; effluent clean-up and process water recycling
Transferases	Transglutaminase (EC 2.3.2.13)	Modification of wool proteins improved fabric strength, dyeability and shrink resistance
	Glycosyltransferase	Potential modification of cellulose fibers for functional textile finishes (e.g., antimicrobial or bioactive coatings)

	Acyltransferase	Enzymatic modification and decolourization of synthetic dyes in coupled oxidation systems (research-scale)
Hydrolases	Amylase (EC 3.2.1.1)	Desizing of starch-based sizing agents from cotton fabrics
	Cellulase (EC 3.2.1.4)	Bio-polishing of cotton, denim finishing, surface fibril removal and improved fabric softness
	Pectinase (EC 3.2.1.15)	Bioscouring of cotton through the removal of pectins and non-cellulosic impurities
	Lipase (EC 3.1.1.3)	Removal of fats, waxes, oils, and spinning lubricants during scouring
	Protease (EC 3.4.21–24)	Wool and silk processing, removal of protein-based impurities, controlled surface modification
Lyases	Pectin lyase (EC 4.2.2.10)	Scouring of cotton by cleaving pectin without water involvement, enabling low-water processing
Ligases	Sortase A (EC 6.3.2.-)	Enzymatic grafting of peptides or functional molecules onto textile surfaces for advanced functional finishes
Isomerases	Protein disulfide isomerase (EC 5.3.4.1)	Potential modification of disulfide bonds in wool and silk fibers to improve handling and shrink resistance (emerging research)
	Xylose isomerase (EC 5.3.1.5)	Conversion of lignocellulosic textile biomass (cotton linters, hemp) in textile-linked biorefinery processes
	Glucose isomerase (EC 5.3.1.18)	Conversion of glucose from starch sizing into value-added sugar derivatives for greener textile auxiliaries (indirect application)

IV. ROLE OF ENZYME ASSAY IN IMMOBILIZATION

Enzyme assays play a crucial role not only in measuring enzyme activity but also in evaluating the effectiveness of enzyme immobilization. These assays help determine how much activity is retained after immobilization, how stable the enzyme remains during repeated use. Assays also help determine whether substrate accessibility is affected. By comparing the activity of free and immobilized enzymes, these assays provide essential information for selecting suitable immobilization strategies and optimizing enzyme-based textile processes. The table below summarizes commonly used assays for textile-relevant enzymes and highlights their importance in immobilized enzyme applications. The assays that can be done to evaluate the efficiency of enzyme immobilization are summarised in Table 2.

Table 2 – Enzyme Assays for evaluating Immobilization Efficiency

Assay	Target enzyme(s)	Textile processing stage
Syringaldazine assay	Laccases	Bleaching / Finishing
Guaiacol assay	Laccases, Peroxidases	Finishing / Effluent treatment
Pyrogallol assay	Peroxidases	Finishing
UV assay (H_2O_2 decomposition)	Catalases	Post-bleaching
Hydroxamate assay	Transglutaminases	Finishing
Fluorometric assay	Glycosyltransferases, Transglutaminases, Acyltransferases	Advanced finishing
Colorimetric assay	Glycosyltransferases	Finishing / Coating
DTNB (Ellman's reagent) assay	Acyltransferases	Finishing
DNS assay	Amylases, Cellulases, Pectinases	Desizing / Scouring / Bio-polishing
CMC assay	Cellulases	Bio-polishing
Viscosity assay	Pectinases	Scouring
p-Nitrophenyl butyrate assay	Lipases	Scouring / Finishing
Titration assay (NaOH)	Lipases	Scouring

Azocasein assay	Proteases	Scouring / Finishing
BCA assay	Proteases	Degumming / Finishing
Thiobarbituric acid (TBA) assay	Pectin lyases	Scouring
Di-E-GSSG fluorescence assay	Protein disulfide isomerase	Finishing
D-xylose → D-xylulose (cysteine–carbazole) assay	Xylose isomerase	Fibre modification (R&D)
HPLC-based product analysis	Glucose isomerase, Xylose isomerase, Acyltransferases	Finishing / R&D
FRET assay	Sortase	Advanced functional finishing

V. MECHANISMS OF IMMOBILIZATION AND CHALLENGES

Adsorption: Adsorption immobilizes enzymes through weak adhesion on a substrate surface. The surface chosen is generally referred to as a carrier. The effectiveness of immobilization is strongly influenced by the carrier and surface modifications. Studies have shown that Lac-PMag (laccase immobilized on magnetic iron oxide particles) can achieve approximately 99% removal of Acid Blue 277 and Acid Black 172 dyes through synergistic adsorption and enzymatic biodegradation. Enzyme adsorption has been reported on supports such as coconut fibres and mesoporous materials via hydrophobic and electrostatic interactions, which can reduce enzyme aggregation and enhance stability. In another study, protease was immobilized on chitosan-blended cellulose monoacetate nanofibers by means of adsorption followed by glutaraldehyde-assisted crosslinking. This process achieved an immobilization yield of 83% by retaining 20–33.5% activity after seven reuse cycles.

However, the challenges of commercializing adsorption-based enzyme immobilization cannot be overlooked. The weak adhesion between enzymes and the supporting carrier can lead to enzyme leaching during textile processing conditions, which involve fluctuations in pH and temperature. In the presence of surfactants, salts, and other auxiliaries, the adsorbed enzymes can lose their adhesion with the supporting material. Additionally, poor orientation on the adsorption surface may block active sites, resulting in reduced catalytic efficiency. Repeated processing cycles can cause activity loss due to conformational changes, enzyme desorption or fouling by textile impurities such as waxes, sizing agents, and residual dyes. This limits the wider textile industrial adoption of adsorption-based immobilized enzymes.

Covalent Bonding: Covalent bonding irreversibly immobilizes enzymes using functional groups such as amino, thiol, carboxyl moieties. This is done by incorporating supports such as silica and chitosan. Recent research report urease immobilization onto a cation-exchange textile using 1-ethyl-3-3-dimethylaminopropyl carbodiimide (EDC), carboxymethyl cellulose and glutaraldehyde, where EDC activation in combination with N-hydroxysuccinimide yielded the highest stability and catalytic performance.

Despite its stability and low enzyme leaching, covalent immobilization presents challenges for large-scale textile wet processing. The irreversible nature of covalent bonding can lead to loss of enzymatic activity if binding occurs near or within the active site. The use of chemical crosslinkers, such as glutaraldehyde, glyoxal, or carbodiimides, may cause toxicity issues and result in higher processing costs due to the need for additional washing steps. This contradicts the sustainability goals of the textile industry. Also, the variety of textile substrates is another concern. Non-uniform distribution of immobilized enzymes and mass-transfer limitations may arise especially in dense or finished fabrics. All these factors, along with difficulties in regenerating or replacing deactivated enzymes on covalently modified textiles, have restricted the industrial adoption of covalent enzyme immobilization in textile wet processing.

Sol-gel method: This method has been reported as a versatile strategy for enzyme immobilization and functional finishing of textiles. Immobilization of papain on cotton fabrics through sol–gel processing has been shown to enhance enzyme pH tolerance and reusability. In another study, manganese peroxidase (MnP) entrapped within hydrophobic sol–gel networks formed from tetramethoxysilane and propyltrimethoxysilane demonstrated increased

stability for decolorization of textile wastewater. Sol–gel method also gives flexibility for adding functionalities on textiles, like water repellency and ultraviolet protection.

Despite the promising outcomes, the wide-scale implementation of sol–gel treatments in the textile industry remains challenging. Major limitations include the relatively costly silane precursors, use of organic solvents, need for acidic/alkaline catalysts. Achieving uniform gel coatings and controlled layer thickness across wide fabric surfaces is also difficult under existing application conditions. Also extended processing and curing durations result in adverse effects on fabric handle. Concerns related to solvent recovery, effluent treatment and occupational safety associated with alkoxysilanes further limit commercialization. This emphasizes the need for further research on environmentally benign water-based sol–gel systems.

Ionic bonding: It is also a reversible enzyme immobilization mechanism based on electrostatic interactions which offers ease of regeneration. Nanomaterials have shown enhanced enzyme durability and recyclability for this kind of enzyme immobilization. Application of green-synthesized nanomaterials supports sustainable biocatalysis. The commercialization in textile wet processing is constrained by the weak nature of ionic bonding that can lead to enzyme leaching under fluctuating pH, temperature and electrolyte conditions which are very common in textile processing.

A major limitation is the difficulty in recovering and reusing enzyme-loaded nanoparticles from large volumes of textile effluents. Recovery of the immobilized enzyme would require energy-intensive filtration, centrifugation, segregation systems which will ultimately increase the operational cost and complexity in textile processes. The loss of nanoparticle loss into wastewater streams further raises environmental and regulatory concerns limiting large-scale industrial adoption.

Entrapment: Another method for immobilizing enzymes is the entrapment method. Recent research reported the application of ginger peroxidase entrapped within guar gum–alginate/agarose hydrogels for textile effluent decolorisation. This process showed enhanced stability and achieved up to 80% textile effluent decolorization over 30 days while retaining 55–68% activity after ten reuse cycles and significantly reducing effluent genotoxicity. There are many other reports for immobilisation using an entrapment strategy for textile effluent remediation. The large-scale adoption of enzyme entrapment in textile wet processing is limited by enzyme leakage from porous matrices, mass-transfer resistance, mechanical instability of gels under agitation, and difficulties in recovering and reusing entrapped biocatalysts in continuous textile processing systems.

Cross-linking and Encapsulation: Polymeric shells and matrices such as alginate or layered nano-films are used for immobilizing enzymes. This kind of encapsulation finds applications in textile effluent treatment and functional finishing. Laboratory-scale results have demonstrated high catalytic efficiency and reusability. The major challenge for adopting this method in the textile industry is the instability of capsules under high shear, which can lead to enzyme leakage during prolonged wet processing. Another challenge is the difficulty in recovering and reusing encapsulated enzymes from huge volumes of effluent. Nevertheless, commercialization is limited due to partial loss of enzyme activity arising from excessive cross-linking.

VI. SUMMARY

Enzyme immobilization offers sustainable benefits for textile industry. Despite the proven laboratory-scale results, the large-scale commercialization of this technique, remains limited due to high immobilization costs, partial loss of activity, enzyme leaching, limited durability under harsh textile conditions, and poor compatibility with existing continuous processing systems highlighted above. Overcoming these techno-commercial challenges through scalable, low-cost immobilization strategies is essential for wider industrial adoption.

Sustainable and biodegradable supports such as sodium alginate, chitosan, carrageenan, polycaprolactone have demonstrated significant potential for enzyme immobilization by enhancing enzyme stability, reusability and eco-friendliness. These findings underscore the importance of biodegradable supports in developing cleaner and more sustainable textile technologies. Further research on enzyme immobilization is necessary which not only limits to lab-scale immobilization but

also focusses on its compatibility in bulk processes of textile mills.

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