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# Immobilization of Phospholipase D on Nanomaterials for Enhanced Biocatalysis in Food Processing: A Survey

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## Abstract

This survey explores the integration of phospholipase D (PLD) with nanomaterials to enhance enzyme stability and efficiency for biocatalysis in food processing. Enzyme immobilization, a cornerstone of biotechnology, addresses the limitations of free enzymes by improving their operational stability and reusability. The application of nanomaterials, such as porous silica and hybrid nanoflowers, offers significant advancements in enzyme stabilization, enhancing reaction specificity, product yield, and process sustainability. This multidisciplinary approach underscores the potential of nanotechnology in optimizing food processing techniques, contributing to improved texture, flavor, and nutritional value of food products. Despite the promising applications, challenges persist in enzyme stability, scalability, and regulatory compliance. This survey categorizes immobilization methods, evaluates the role of nanocarriers, and highlights the importance of co-immobilization strategies. Future research should focus on developing versatile, eco-friendly immobilization strategies, optimizing enzyme-substrate interactions, and addressing regulatory challenges to fully exploit the potential of immobilized enzymes. By advancing these areas, the field can continue to offer sustainable and efficient solutions to meet the demands of modern food processing.

## 1 Introduction

### 1.1 Enzyme Immobilization in Biotechnology

Enzyme immobilization is fundamental to modern biotechnology, enhancing enzyme stability and functionality critical for industrial applications [1]. This technique mitigates the limitations of free enzymes, such as instability and environmental susceptibility, by improving operational stability and reusability. The integration of advanced materials, particularly nanomaterials, has been instrumental in this enhancement, providing tailored solutions for improved enzyme performance [2]. For example, porous silica doping has been shown to enhance microrobots' performance in nonideal environments, illustrating the versatility of immobilization techniques across various applications [3].

Recent surveys have outlined prevalent approaches for enzyme stabilization, emphasizing practical case studies that address existing literature gaps [2]. These studies highlight the necessity of optimizing immobilization protocols to overcome common challenges and improve biocatalytic process effectiveness [4]. Biocatalysis plays a pivotal role in sustainable industrial chemistry, contributing to resource-efficient manufacturing practices that offer greener alternatives to traditional chemical methods.

The scope of enzyme immobilization transcends mere efficiency enhancement, encompassing the discovery and application of novel biocatalysts through innovative methodologies. Advances in biocatalysis, driven by multidisciplinary technologies like artificial intelligence, automation, and ultra-high-throughput methods, are vital for addressing the complexities of modern industrial processes

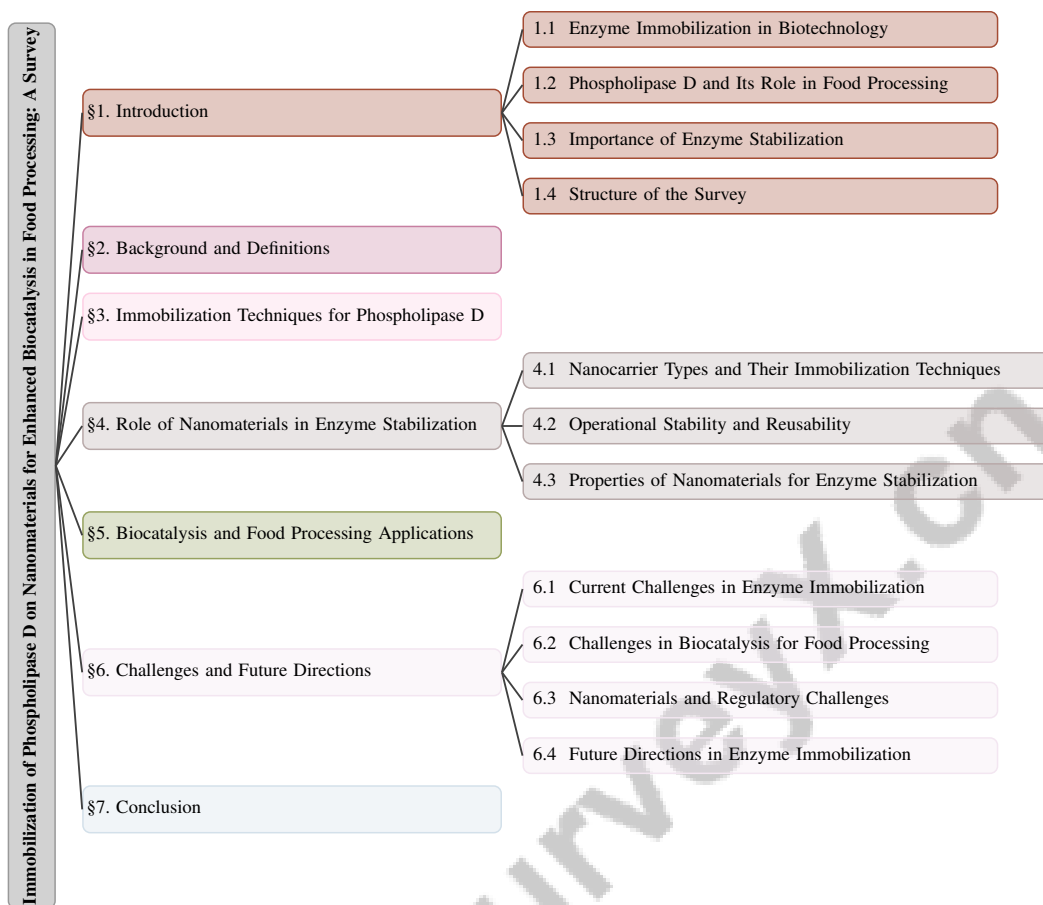


Figure 1: chapter structure

and enhancing sustainability. This evolution not only fosters the development of robust biocatalysts and efficient enzymatic pathways but also aligns with global sustainability initiatives, addressing economic and ecological challenges across sectors such as pharmaceuticals and specialty chemicals [5, 6, 7, 8]. By incorporating cutting-edge approaches, enzyme immobilization remains crucial to biotechnological advancements.

## 1.2 Phospholipase D and Its Role in Food Processing

Phospholipase D (PLD) is a vital enzyme in food processing, catalyzing the hydrolysis of phospholipids into phosphatidic acid and free head groups, thus altering the structural and functional properties of food components. This enzymatic activity is essential for enhancing food texture and flavor and for developing functional foods with improved nutritional profiles [9]. The use of PLD in forming hybrid nanoflowers (HNFs) exemplifies its utility, as these structures facilitate the enzymatic hydrolysis of food proteins, improving their solubility and emulsifying properties [10].

Characterization of the PLD gene family in various plant species, including allotetraploid peanut and its diploid progenitors, sheds light on the enzyme's role in plant growth and abiotic stress responses, which is crucial for optimizing food production and processing under stress conditions [11]. Additionally, PLD's involvement in stress responses in crops like sorghum emphasizes its potential applications in enhancing food product stability and quality [12].

Integrating PLD into nanotechnological applications provides a promising strategy for advancing food preservation and processing techniques, addressing knowledge gaps regarding various nanomaterials' efficacy in functional food development while meeting the industry's demand for improved enzyme productivity and extended shelf life [13]. The immobilization of PLD on nanomaterials offers distinct

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advantages over free enzymes, including enhanced stability and reusability, which are critical for industrial applications [14].

Furthermore, exploring nanotechnology applications in the food industry underscores the increasing role of nanoparticles in food processing, packaging, safety, and nutritional enhancement [15]. This is complemented by the growing interest in biopolymers as sustainable alternatives to synthetic materials, contributing to more sustainable food processing practices [16]. The utilization of PLD not only enhances the functional properties of food components but also supports the industry's sustainability and efficiency goals [8].

### 1.3 Importance of Enzyme Stabilization

Enzyme stabilization is critical for enhancing the efficiency and cost-effectiveness of biocatalytic processes, addressing the instability and high costs associated with free enzymes in industrial applications. The challenge of enzyme instability is exacerbated by harsh industrial conditions, which can significantly diminish enzymatic activity and functionality [2]. Effective immobilization techniques are essential for overcoming these limitations, enabling enzyme reuse and improving operational efficiency [17].

Recent advancements in enzyme immobilization have concentrated on key issues such as substrate specificity and scalability, which are vital for successful biocatalysis integration into large-scale industrial processes [5]. Poorly designed immobilization protocols can lead to suboptimal outcomes, limiting the full potential of enzyme technologies [4]. To address these challenges, innovative approaches enabling real-time monitoring of enzymatic reactions have been proposed, offering solutions to obstacles such as water absorbance during reactions [18].

Developing robust enzyme stabilization strategies enhances biocatalytic performance and contributes to the sustainability of industrial practices by minimizing waste and energy consumption. As the field evolves, integrating advanced materials and technologies promises to further improve enzyme stability and broaden biocatalysis applicability across diverse sectors [2].

### 1.4 Structure of the Survey

This survey comprises several key sections, each addressing specific aspects of phospholipase D immobilization on nanomaterials for enhanced biocatalysis in food processing. The introductory section provides a comprehensive overview of enzyme immobilization, focusing on phospholipase D's role in food processing and the significance of enzyme stabilization. This foundation sets the stage for subsequent sections that delve into core concepts and methodologies.

The second section, Background and Definitions, offers a detailed examination of fundamental concepts such as enzyme immobilization, phospholipase D, nanomaterials, and biocatalysis, emphasizing their importance in food processing applications. This foundational knowledge is crucial for understanding advanced topics discussed later.

The third section explores various immobilization techniques for phospholipase D, categorizing and describing different methods, including novel technologies and co-immobilization concepts. This section highlights recent advancements and evaluates the advantages and disadvantages of each approach.

The fourth section examines the role of nanomaterials in enzyme stabilization, focusing on their properties that make them suitable for this purpose. It discusses different types of nanocarriers, their immobilization techniques, and factors affecting the operational stability and reusability of immobilized enzymes.

The fifth section investigates the applications of immobilized phospholipase D in food processing, demonstrating how this approach improves reaction specificity, product yield, and process sustainability. It provides examples of specific food processing applications and the role of nanotechnology in advancing food science.

The penultimate section addresses challenges and future directions in enzyme immobilization and biocatalysis, identifying current obstacles and suggesting potential research directions and technological advancements to overcome these challenges. It also examines regulatory challenges associated with using nanomaterials in enzyme stabilization.

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The survey concludes by summarizing key findings discussed throughout the document and emphasizing the significant potential of immobilized phospholipase D in advancing nanomaterial development, enhancing biocatalysis in food processing. This innovation improves enzyme stability and reusability and facilitates efficient product recovery, optimizing industrial applications in food technology and potentially revolutionizing enzymatic processes [14, 17]. Suggestions for future research are also provided to guide ongoing advancements in this multidisciplinary field. The following sections are organized as shown in Figure 1.

## **2 Background and Definitions**

### **2.1 Nanomaterials**

Nanomaterials, characterized by structural components under 100 nanometers, offer unique advantages in enzyme immobilization, categorized into carbon-based, metal-based, and other types. Carbon-based nanomaterials like fullerenes, carbon nanotubes, and graphene are renowned for their electrical conductivity, mechanical strength, and large surface areas, making them ideal for stable enzyme-nanomaterial composites [7]. Computational studies have further elucidated their interactions with proteins, highlighting their potential in enzyme stabilization [19].

Metal-based nanomaterials, particularly metal oxide nanoparticles, are noted for their high surface area-to-volume ratios and catalytic properties, enhancing enzyme activity and stability [1]. The shape of these nanoparticles, such as cup, disc, and rod, influences their biological interactions, crucial for optimizing enzyme immobilization [20]. Magnetic nanoparticles facilitate the easy recovery and reuse of immobilized enzymes, improving process efficiency [17].

Other nanomaterials, including magnetic nanoparticles and nanofibers, also enhance enzyme immobilization. Rice bran-derived nanofibers, for instance, offer high surface area and porosity advantageous for enzyme encapsulation [21]. The use of porous silica in microrobots demonstrates nanomaterials' role in mechanical stability and adhesion, relevant to enzyme immobilization [3].

Nanomaterials are further classified as natural, incidental, bioinspired, and engineered, each with specific benefits and limitations [22]. Biopolymers, a renewable and biodegradable subset, are suitable for environmentally friendly applications [16]. In food science, nanomaterials enhance processing, packaging, and preservation, contributing to functional foods with superior nutritional and sensory properties [13]. The diverse nanomaterials and their tailored properties underscore their potential in advancing enzyme immobilization technologies, particularly for biocatalytic processes in food processing.

### **2.2 Biocatalysis**

Biocatalysis employs natural catalysts, primarily enzymes, to facilitate chemical reactions under mild conditions, offering a sustainable alternative to traditional chemical synthesis [23]. This method is pivotal in industrial applications, enhancing chemical production efficiency while addressing environmental challenges and meeting the demand for sustainable practices.

In food processing, biocatalysis enables precise substrate transformations, improving texture, flavor, and nutritional value, thus optimizing production processes and supporting functional food development [24]. Recent advancements focus on designing enzymatic pathways for complex natural product synthesis, addressing challenges like substrate specificity and scalability [5]. Computational studies provide insights into enzyme-substrate interactions, crucial for optimizing biocatalytic processes, similar to strategies in other industrial applications like the Dynamic Resource Allocation Algorithm (DRAA) [25].

Innovative technologies for monitoring enzymatic reactions, such as Terahertz BioMEMS, exemplify progress in biocatalysis by enabling real-time tracking of reaction dynamics, enhancing process control and efficiency [18]. The ongoing evolution of biocatalysis, driven by advancements in enzyme technology and process optimization, promises to broaden its applicability across diverse sectors, particularly in food processing, improving product quality and sustainability.

## 2.3 Significance in Food Processing

The integration of enzyme immobilization and nanomaterials significantly enhances food processing by improving efficiency, product quality, and sustainability. Immobilization optimizes biocatalytic reactions, particularly in flow systems, enhancing productivity and enabling continuous processing [6]. By stabilizing enzymes and improving reusability, these techniques reduce process costs and complexity, aligning with industry demands for cost-efficient systems [8]. This approach also supports integrating biocatalysis into conventional chemical processes, promoting sustainable industrial practices [23].

Nanotechnology advances food processing by offering solutions for food safety, shelf life extension, and nutritional delivery [15]. Nanomaterials enhance enzyme stability and activity, facilitating diverse food processing applications. They optimize enzyme-substrate compatibility, expanding biocatalysis scope [24]. However, challenges persist in standardizing definitions and regulations for nanomaterials, alongside concerns about toxicity and environmental impact [22].

Microbial enzymes immobilized on nanomaterials improve food quality, safety, and processing efficiency, enhancing sensory and nutritional properties while supporting sustainability goals by reducing waste and energy consumption [9]. As the field progresses, integrating enzyme immobilization and nanotechnology is poised to revolutionize food processing, offering innovative solutions to meet the food industry's evolving demands.

In recent years, the field of enzyme immobilization has gained significant attention due to its implications for biocatalytic applications. A comprehensive understanding of the various techniques available is essential for optimizing enzyme performance. As illustrated in Figure 2, the hierarchical structure of immobilization techniques for phospholipase D is categorized into physical and chemical methods, novel technologies, and co-immobilization strategies. This figure not only highlights the key methods employed in enzyme immobilization but also delineates their respective benefits and challenges. Moreover, it underscores the future directions in the field, particularly the integration of advanced materials and green chemistry principles aimed at enhancing biocatalytic efficiency and sustainability. Such an integrative approach is crucial for the development of more effective and environmentally friendly biocatalytic processes.

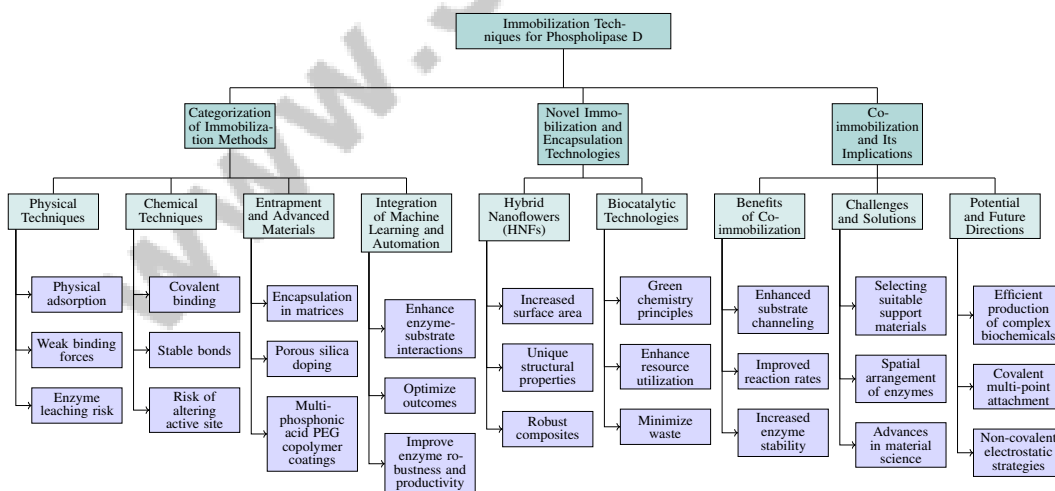


Figure 2: This figure illustrates the hierarchical structure of immobilization techniques for phospholipase D, categorized into physical and chemical methods, novel technologies, and co-immobilization strategies. It highlights the key methods, benefits, challenges, and future directions in enzyme immobilization, emphasizing the integration of advanced materials and green chemistry principles to enhance biocatalytic efficiency and sustainability.

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### 3 Immobilization Techniques for Phospholipase D

#### 3.1 Categorization of Immobilization Methods

The immobilization of phospholipase D (PLD) is crucial for enhancing its stability and functionality in biocatalytic applications, especially in food processing. Immobilization methods are categorized into physical and chemical techniques, each influencing enzyme stability and application [2, 8]. Physical adsorption uses weak binding forces like van der Waals, hydrogen bonds, or hydrophobic interactions, which simplify the process but may lead to enzyme leaching due to lower binding strength [14]. Conversely, covalent binding forms stable bonds between the enzyme and carrier, improving stability and resistance to desorption, albeit with a risk of altering the enzyme's active site [26].

Entrapment involves encapsulating the enzyme within a matrix, such as gels or polymers, which allows substrate access while limiting enzyme mobility. This protects against environmental changes but may impede substrate diffusion, affecting reaction rates [14]. Techniques like porous silica doping enhance the stability of magnetic soft microrobots, offering insights for PLD immobilization strategies [3]. Advanced materials, such as multi-phosphonic acid PEG copolymer coatings, improve nanoparticle stability, which can be leveraged in immobilization strategies [1]. Engineered enzymes catalyze reactions more efficiently, aligning with immobilization goals by minimizing waste [23].

Integrating machine learning and automation into enzyme immobilization enhances enzyme-substrate interactions and optimizes outcomes, addressing challenges of poorly designed protocols. Data-driven techniques refine immobilization methods, improving enzyme robustness and productivity, and facilitating easier recovery and reuse in industrial applications [4, 14]. This categorization provides a comprehensive framework for selecting suitable PLD immobilization techniques based on specific application requirements.

#### 3.2 Novel Immobilization and Encapsulation Technologies

Recent advancements in immobilization and encapsulation technologies have significantly enhanced enzyme stability and functionality, enabling continuous biocatalysis in industrial applications [27]. Enzymatic hydrolysis is a cutting-edge approach that breaks down food proteins into smaller polypeptides, facilitating the formation of hybrid nanoflowers (HNFs) [10]. These HNFs, with increased surface area and unique structural properties, serve as innovative platforms for enzyme immobilization, improving enzyme activity and stability.

The integration of inorganic materials with bioactive components in HNFs creates robust composites that optimize the immobilization process and expand enzyme applications in food processing. This approach enhances solubility and emulsifying properties, crucial for food science and biocatalysis. The exceptional loading capacities of HNFs for sensitive natural pigments indicate their potential as effective delivery systems across industries, including food, cosmetics, and pharmaceuticals [27, 4, 10, 23].

The advancement of biocatalytic technologies, driven by molecular biology and biotechnology breakthroughs, is pivotal for creating environmentally friendly industrial processes aligned with green chemistry principles. These innovations enhance resource utilization and minimize waste across sectors, such as pharmaceuticals and specialty chemicals [5, 23, 8]. By leveraging hybrid structures' unique properties, researchers are overcoming traditional limitations in enzyme immobilization, promising to expand its applicability in enhancing food processing efficiency and sustainability.

#### 3.3 Co-immobilization and Its Implications

Co-immobilization involves the simultaneous immobilization of multiple enzymes on a single support, enhancing the efficiency and versatility of biocatalytic processes. This method addresses the complexity of enzyme interactions within multi-enzyme systems, influencing overall performance and stability [28]. Co-immobilizing enzymes can create synergistic effects that improve reaction rates and product yields, particularly in cascade reactions where one enzyme's product serves as another's substrate.

Benefits of co-immobilization include enhanced substrate channeling, reducing diffusion limitations and increasing reaction efficiency, crucial for industrial applications prioritizing time and resource

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efficiency. This method also enhances enzyme stability by creating a supportive microenvironment that promotes activity and protects against denaturation. Proximity facilitates optimal interactions among enzymes, leading to improved thermal and operational stability under extreme conditions [27, 14, 4, 26].

Implementing co-immobilization poses challenges, particularly in selecting suitable support materials that accommodate multiple enzymes while maintaining their activity and stability. Careful consideration of immobilization techniques and support properties is essential, as suboptimal choices can hinder enzyme performance. Advances in material science and bioprocess engineering may provide innovative solutions, highlighting the need for ongoing research [27, 4, 14]. The spatial arrangement of enzymes is crucial; improper orientation can lead to steric hindrance and reduced efficiency. Advanced materials and techniques, such as nanocarriers and hybrid nanostructures, offer promising solutions by providing customizable platforms tailored to specific enzymatic needs.

Co-immobilization holds significant potential for advancing biocatalysis, enabling efficient and sustainable production of complex biochemicals. Ongoing research into optimal conditions and innovative materials is pivotal in advancing biocatalytic processes across industries. Recent advancements, including covalent multi-point attachment and non-covalent electrostatic strategies, enhance biocatalyst stability and reusability, facilitating continuous flow synthesis. By integrating these developments with green chemistry principles, co-immobilization is expected to improve biocatalytic reaction efficiency and contribute to the sustainable production of pharmaceuticals and fine chemicals, marking a significant shift in industrial bioprocessing practices [4, 27, 14, 23].

## **4 Role of Nanomaterials in Enzyme Stabilization**

### **4.1 Nanocarrier Types and Their Immobilization Techniques**

Nanocarriers are pivotal in enzyme immobilization, enhancing stability, reusability, and catalytic efficiency across industrial applications [17]. They are categorized by origin—natural, incidental, bioinspired, and engineered—and by dimensionality—0D, 1D, 2D, and 3D [22], each offering unique properties for enzyme immobilization, thus expanding biocatalysis applications, especially in food processing.

Carbon-based nanomaterials, such as fullerenes, carbon nanotubes, and graphene, are prized for their electrical conductivity and mechanical strength, forming stable enzyme-nanomaterial composites [19]. Computational studies optimize enzyme interactions with these materials, enhancing stabilization. Similarly, metal-based nanomaterials like gold nanorods and nanoclusters possess catalytic properties that bolster enzyme activity and stability [19].

Polymer-based nanocarriers are favored for their versatility, engaging in diverse interactions with enzymes. The selection of polymer supports is crucial, as demonstrated by the classification of methods based on interaction types [26]. Techniques such as ATRP brush growth on nanocarrier surfaces deepen understanding of these carriers' properties and their role in enzyme stabilization [20].

Inorganic nanocarriers, including porous silica, are noted for enhancing the stability and reusability of immobilized enzymes due to their high surface area and structural properties, aiding enzyme encapsulation akin to stabilizing microrobots [3]. Enzymatic hydrolysis improves nucleation sites in food proteins, facilitating hybrid nanoflower (HNF) formation for enzyme immobilization [10].

Nanocarriers advance catalytic activity and operational efficiency while enhancing food safety and quality through their unique properties [13]. Continued research into optimal conditions and materials underscores nanocarriers' significance in advancing biocatalytic processes across various sectors.

As shown in Figure 3, nanomaterials are integral to enzyme stabilization, utilizing their unique properties to enhance stability and functionality. The first image illustrates a continuous flow chemistry system, highlighting the integration of synthetic biocatalysis. The second diagram shows electron transfer mechanisms enabled by nanomaterials, demonstrating their potential in antimicrobial strategies. The third image depicts nanoparticle size distribution, emphasizing precision in synthesis crucial for optimizing enzyme immobilization. These examples underscore nanomaterials' versatility and transformative potential in enhancing enzyme stability and function across industrial and biomedical applications [27, 29, 1].

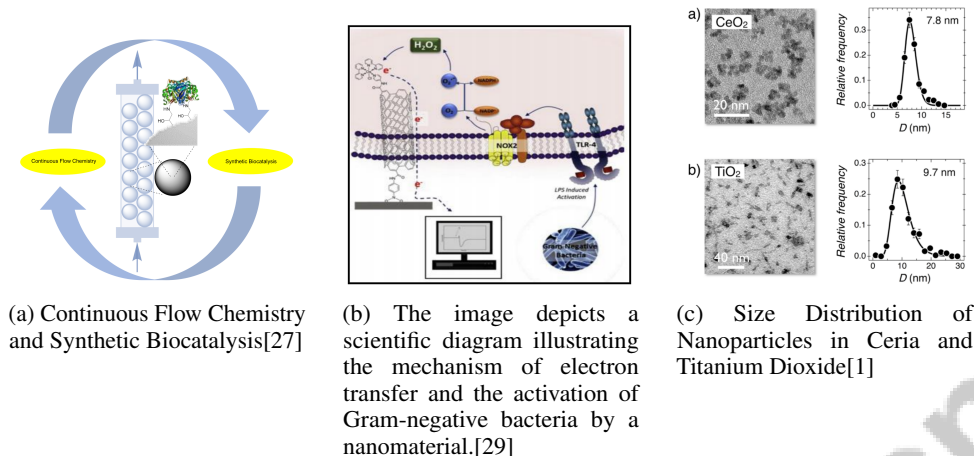


Figure 3: Examples of Nanocarrier Types and Their Immobilization Techniques

## 4.2 Operational Stability and Reusability

Operational stability and reusability are critical for industrial enzyme applications, particularly in continuous processes. Immobilization enhances enzyme stability by creating a protective environment that mitigates adverse effects from extreme conditions, preserving catalytic activity over time [14].

Reusability is a major advantage, allowing repeated use across multiple cycles, reducing costs and improving efficiency [2]. The immobilization technique and support material choice are vital, with covalent binding and entrapment offering higher reusability than physical adsorption due to stronger interactions [4].

Nanomaterials as supports enhance both stability and reusability. Their high surface area and unique properties facilitate robust enzyme binding, minimizing leaching and maintaining activity over multiple cycles [1]. Magnetic nanoparticles enable easy enzyme recovery, further improving reusability [17].

Hybrid nanoflower structures significantly improve enzyme stability by providing a conducive microenvironment that supports enzyme conformation and enhances resistance to denaturing agents [10]. Advances in immobilization technologies promise further enhancements in stability and reusability, broadening their industrial applicability.

## 4.3 Properties of Nanomaterials for Enzyme Stabilization

Nanomaterials' unique properties are crucial for enzyme stabilization in biocatalytic applications. Their high surface area-to-volume ratio enhances enzyme loading capacity and facilitates strong interactions essential for maintaining enzyme conformation and activity under diverse conditions [1]. For example, nanofiber-encapsulated enzymes like phytase exhibit enhanced thermal stability [21].

Multifunctional nanomaterials, including copolymers, stabilize metal oxide nanoparticles in biological environments, preserving enzyme activity under challenging conditions [1]. These copolymers form protective matrices that shield enzymes from denaturing agents, ensuring sustained catalytic performance. The structural diversity of nanomaterials allows for tailored enzyme-support interactions, optimizing stability and activity for specific applications.

Integrating nanomaterials in enzyme stabilization leverages unique properties such as magnetic responsiveness and electrical conductivity, facilitating easy enzyme recovery, enhancing reusability, and contributing to operational efficiency. Research into genetic characterization of enzymes, like SbPLD genes in plants, highlights nanomaterials' potential in enhancing enzyme functionality and stability under stress [12].

Nanomaterials' unique characteristics, including structural diversity, high surface area, and functionalization potential, make them effective platforms for enzyme stabilization. These properties not only enhance enzyme stability and reusability across various applications but also drive significant advance-



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ments in biocatalysis and biotechnology. Recent studies affirm the effectiveness of nanocarriers, from carbon nanotubes to superparamagnetic nanoparticles, in enhancing enzyme performance through immobilization, thus extending operational lifespan and efficiency under challenging conditions [19, 26, 17].

## **5 Biocatalysis and Food Processing Applications**

### **5.1 Applications of Nanocarriers in Enzyme Reusability**

Nanocarriers are pivotal in enhancing enzyme reusability, a key factor in the cost-effectiveness and efficiency of biocatalytic processes within food processing. By facilitating enzyme immobilization, nanocarriers augment operational stability, enabling repeated enzyme use across multiple reaction cycles, thus reducing overall costs and promoting sustainability [5]. Their high surface area-to-volume ratio increases enzyme loading capacity, essential for maintaining catalytic efficiency over extended periods, which boosts the yield and quality of food products by supporting efficient biochemical reactions under diverse industrial conditions [4, 26, 2, 9]. The physicochemical properties of nanocarriers also minimize enzyme leaching and degradation, extending their usability in industrial settings. Recent advancements in artificial intelligence and automation have optimized enzymatic pathways, enhancing process efficiency by allowing precise control of reaction conditions and maximizing enzyme performance [5]. Tailoring nanocarrier properties to specific enzymatic needs underscores their transformative potential in food processing, addressing evolving industry demands.

### **5.2 Enzyme Immobilization and Hybrid Nanoflowers**

Hybrid nanoflowers (HNFs) represent a significant advancement in enzyme immobilization technologies, providing a novel platform to enhance enzyme stability and activity in food processing. With their expansive surface area, HNFs facilitate efficient enzyme loading and stabilization, improving catalytic performance [10]. Their ability to stabilize natural pigments extends their applicability to cosmetics and medicine. HNFs, formed by integrating inorganic components with bioactive substances, optimize enzyme immobilization processes, enhancing enzyme activity and improving solubility and emulsifying properties critical for food processing. Their structural properties support durable composites that preserve enzyme activity under various conditions, significantly enhancing food product stability and shelf life. For instance, incorporating enzymatically hydrolyzed proteins in HNF formation improves loading capacities for sensitive pigments like Monascus red and curcumin, while encapsulating thermally unstable enzymes such as phytase within rice bran nanofibers increases thermal stability, sustaining enzymatic activity at elevated temperatures [4, 10, 21, 2, 23]. The innovative use of HNFs aligns with industry demands for sustainable and efficient processing techniques, overcoming traditional immobilization challenges by enhancing substrate accessibility and enzyme reusability, thus optimizing biocatalytic performance across sectors from pharmaceuticals to food processing [4, 14].

### **5.3 Nanotechnology in Food Science**

Nanotechnology plays a crucial role in advancing food science, particularly through innovative enzyme immobilization techniques. The integration of advanced nanomaterials has significantly enhanced the sensitivity and selectivity of nanosensors, essential for monitoring and optimizing food processing operations [30]. These nanosensors enable real-time tracking of enzymatic reactions, improving process control and ensuring high-quality food production. Nanomaterials, with their high surface area-to-volume ratios and versatile functionalization capabilities, facilitate effective enzyme immobilization, enhancing stability and activity under various processing conditions. This is particularly beneficial in food applications, where maintaining enzyme functionality is vital for product quality and shelf life. Nanomaterials create protective matrices that shield enzymes from denaturing agents, enhancing stability and reusability, which contributes to the overall sustainability of industrial applications, especially in pharmaceuticals and other commodities. The advancements in enzyme immobilization techniques utilizing nanocarriers, such as carbon nanotubes and superparamagnetic nanoparticles, further promote robust enzymatic transformations tailored to specific operational parameters, fostering a more sustainable biobased economy [19, 26, 23, 17]. Nanotechnology's application in enzyme immobilization aligns with industry needs for innovative solutions that enhance food safety, nutritional value, and sensory attributes. Nanomaterials' ability to interact with biological

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entities and improve enzyme-substrate compatibility broadens biocatalysis's scope, facilitating the development of functional foods with enriched nutritional profiles. As research into nanotechnology in food science progresses, its significance in enhancing enzyme immobilization technologies is expected to grow, leading to innovative advancements in the field. Nanocarriers, such as carbon nanotubes and superparamagnetic nanoparticles, are being explored for improving enzyme stability, efficiency, and reusability across various industrial applications, ultimately enhancing food quality and safety by extending shelf life and preventing contamination. The integration of nanotechnology in enzyme applications is poised to drive further developments in food processing, packaging, and overall food quality, showcasing its transformative potential in the food industry [13, 17].

## **6 Challenges and Future Directions**

Advancing enzyme immobilization technologies requires addressing challenges that hinder progress, particularly regarding enzyme stability, activity, and the integration of nanomaterials. These challenges must be thoroughly examined to devise innovative solutions that enhance enzyme technologies across various industrial sectors.

### **6.1 Current Challenges in Enzyme Immobilization**

Enzyme immobilization is limited by several challenges, including enzyme instability, which reduces catalytic efficiency and necessitates system replacements when enzymes become inactive [9]. Maintaining enzyme activity under nonideal conditions is difficult, as external disturbances can impair performance, exemplified by microrobots [3]. Nanomaterial integration introduces additional issues like protein corona formation and nanoparticle aggregation, affecting stability [1]. High production costs, potential enzyme activity loss from leaching or denaturation, and challenges in reproducibility and scalability of nanomaterial synthesis further complicate applications [19, 25]. Magnetic nanoparticle characterization is problematic due to external magnetic fields altering sample states, complicating suitability assessments for enzyme immobilization [31]. Computational bottlenecks from high dimensionality and data volume also pose challenges [25]. A multidisciplinary approach combining material science, biotechnology, and computational methods is essential to enhance enzyme immobilization stability, scalability, and efficiency, maximizing benefits across diverse industrial applications, particularly in food processing and biocatalysis [4, 14, 27, 26, 23].

### **6.2 Challenges in Biocatalysis for Food Processing**

Biocatalysis in food processing faces challenges such as enzyme instability under industrial conditions, affecting activity and efficiency. Enzymes are sensitive to temperature, pH, and inhibitors, impacting performance [2]. Robust immobilization techniques are crucial for stability and functionality. Scalability is another challenge, as maintaining consistent enzyme activity and stability across larger systems is difficult [8]. High enzyme production and immobilization costs necessitate cost-effective methods for stabilization and reuse [26]. Nanotechnology integration offers solutions but introduces challenges like nanoparticle aggregation, potential toxicity, and environmental impacts [22]. Regulatory challenges require comprehensive risk assessments and compliance with food safety standards [15]. Optimizing enzyme-substrate interactions is critical for efficiency, but the dynamic nature of food matrices complicates these interactions [6]. Advanced computational tools and machine learning models offer insights into enzyme-substrate compatibility, guiding effective biocatalytic system design [24]. A multidisciplinary approach integrating enzyme technology, nanomaterials, and computational modeling is necessary to enhance biocatalysis efficiency in the food industry.

### **6.3 Nanomaterials and Regulatory Challenges**

Nanomaterial incorporation in enzyme stabilization presents regulatory challenges that must be addressed for safe industrial application. Long-term effects on health and the environment require rigorous research to inform safety guidelines [22]. Nanomaterials' unique properties enhance enzyme immobilization but introduce variability challenges affecting reliability [29]. Regulatory hurdles and the absence of standardized frameworks create uncertainty, potentially stifling innovation. Establishing robust guidelines and compliance standards is essential for safe nanomaterial use, ensuring they meet safety requirements [22]. A collaborative approach involving researchers, industry

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stakeholders, and regulatory bodies is necessary to address these challenges, enhancing enzyme stabilization and industrial processes [5, 7, 14].

#### 6.4 Future Directions in Enzyme Immobilization

Future research should focus on developing versatile, eco-friendly strategies for enzyme immobilization, emphasizing polymer materials and economic aspects. Sustainable synthesis methods for nanomaterials are crucial for enhancing biocompatibility and exploring emerging applications [22]. Improving synthesis techniques for higher yields and quality while addressing environmental and health concerns is key [7]. Advancements in co-immobilization techniques, particularly involving artificial cells, could enhance enzyme performance [5]. Optimizing enzymatic hydrolysis for food proteins may improve hybrid nanoflower fabrication, presenting new immobilization avenues [10]. Research should aim to embed proteins and compounds to enhance enzyme-substrate compatibility predictions [24]. Cost-effective nanocarrier solutions and exploration of new materials, alongside understanding enzyme-nanocarrier interactions, are essential for performance optimization [17]. Functional studies of candidate PLD genes should explore roles in stress signaling and crop improvement [11]. Establishing regulatory guidelines for nanotechnology in food, coupled with consumer education about nanofoods and exploring safe nanomaterials, will advance the field [13]. Enhancing machine learning's predictive capabilities in enzyme design, exploring new enzymatic pathways, and developing robust biocatalysts are promising research areas [5]. Optimizing BioMEMS design and exploring applications in enzymatic kinetics and biochemical processes are potential exploration areas [18]. Integrating functional groups in copolymers to enhance stability and target biological interactions presents innovation opportunities [1]. Functional analyses of SbPLD genes and regulatory networks in response to environmental stresses could provide enzyme application innovations [12]. Addressing safety and regulatory issues, exploring nanotechnology's environmental implications, and developing effective nanotechnology-based food solutions are critical research areas [15]. Optimizing material properties, such as porous silica, to enhance microrobot and enzyme immobilization performance represents a promising research direction [3].

### 7 Conclusion

This survey underscores the pivotal role of enzyme immobilization in augmenting the stability and reusability of phospholipase D (PLD), particularly through the incorporation of nanomaterials, which significantly propels biocatalysis within food processing. The exploration of various immobilization techniques demonstrates marked enhancements in operational efficiency and cost-effectiveness across diverse sectors, highlighting the importance of tailored approaches for distinct applications. The selection of nanocarriers is crucial in optimizing enzyme performance and boosting bioprocess efficiencies, reflecting the growing relevance of nanotechnology in industrial enzyme applications. However, challenges persist in maintaining enzyme activity and uniformity, necessitating further investigation into novel materials and methodologies to fully exploit the potential of immobilized enzymes.

The integration of PLD with nanomaterials offers promising prospects for enhancing reaction specificity, product yield, and process sustainability in food processing. The unique properties of nanomaterials, such as extensive surface area and adaptable functionalization capabilities, provide a solid foundation for enzyme stabilization, thereby expanding the horizons of biocatalysis in the food industry. Future research should focus on developing innovative immobilization strategies that address current limitations, exploring new varieties of nanomaterials, and optimizing enzyme-nanocarrier interactions to improve overall performance. Additionally, assessing the regulatory and environmental impacts of nanotechnology in food applications will be vital for its wider adoption. By advancing these areas, the field can progress towards sustainable and efficient solutions that cater to the evolving needs of modern food processing.

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