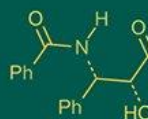


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(6): 1265-1272
www.biochemjournal.com
Received: 08-03-2026
Accepted: 13-04-2026

Sandeep Kumar
Ph.D. Scholar, Department of
Livestock Products
Technology, College of
Veterinary and Animal
Science, Rajasthan University
of Veterinary and Animal
Sciences, Bikaner, Rajasthan,
India

Gagan Chawla
Ph.D. Scholar, Animal
Physiology Division, ICAR-
National Dairy Research
Institute, Karnal, Haryana,
India

Abhishek Tiwari
Ph.D. Scholar Animal
Nutrition Division, ICAR-
National Dairy Research
Institute, Karnal, Haryana,
India

Vinay Kumar
Ph.D. Scholar, Department of
Veterinary and Animal
Husbandry Extension
Education, College of
Veterinary and Animal
Science, Rajasthan University
of Veterinary and Animal
Sciences, Bikaner, Rajasthan,
India

Corresponding Author:
Gagan Chawla
Ph.D. Scholar, Animal
Physiology Division, ICAR-
National Dairy Research
Institute, Karnal, Haryana,
India

Application of immobilized lactase in lactose-free dairy product production: A review

Sandeep Kumar, Gagan Chawla, Abhishek Tiwari and Vinay Kumar

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6p.8884>

Abstract

Lactose intolerance affects approximately 68-70% of the global adult population and has created a rapidly expanding market for lactose-free dairy products, which was valued at USD 12.87 billion in 2024 and is projected to reach USD 19.37 billion by 2029. Industrial production of lactose-free milk, yogurt, cheese, ice cream, and infant formula predominantly relies on enzymatic hydrolysis of lactose by β -galactosidase (lactase; EC 3.2.1.23). However, free lactase is limited by poor thermal stability, high operational costs, and inability to be reused. Enzyme immobilization encompassing adsorption, covalent binding, entrapment, encapsulation, and cross-linking has emerged as the most viable strategy to overcome these constraints. Immobilized lactase systems offer superior stability, reusability over multiple cycles, and compatibility with continuous bioreactor configurations. This review critically examines the principles and techniques of lactase immobilization, the spectrum of support materials from conventional polymers to cutting-edge nanomaterials, industrial applications across diverse dairy product categories, and recent advances including electrospun nanofibers, magnetic nanoparticle-based systems, multi-enzyme cascades, and AI-assisted process optimization. Challenges related to mass transfer resistance, regulatory compliance, and scale-up economics are discussed alongside future perspectives.

Keywords: Lactase, β -galactosidase, enzyme immobilization, lactose-free dairy, nanotechnology

1. Introduction

Lactose (4-O- β -D-galactopyranosyl-D-glucopyranose) is the predominant carbohydrate in mammalian milk, constituting approximately 4.5-5.0% (w/v) of bovine milk and providing nearly 30% of its energy content. Its digestion in the small intestine requires the brush-border enzyme lactase-phlorizin hydrolase (lactase; β -galactosidase; EC 3.2.1.23), which cleaves the β -1,4-glycosidic bond to yield glucose and galactose. Individuals with insufficient intestinal lactase activity are unable to hydrolyze lactose adequately, undigested lactose passes into the colon, where resident microbiota ferment it to short-chain fatty acids, hydrogen, carbon dioxide, and methane, producing characteristic symptoms of bloating, flatulence, diarrhoea, and abdominal cramps collectively termed lactose intolerance (Li *et al.*, 2023) ^[10].

The condition is remarkably prevalent. A systematic review standardized for country size estimated the global prevalence of lactose malabsorption at 68% with regional variation ranging from approximately 28% in Western Europe to over 70% in East Asia and the Middle East (Storhaug *et al.*, 2017) ^[16]. In the Indian subcontinent and Southeast Asia, rates exceed 80-90%, making lactose intolerance a major nutritional concern in countries with high dairy consumption. Lactase activity typically declines after weaning a phenomenon termed lactase non-persistence as a result of epigenetic silencing of the LCT gene encoding lactase, with the onset varying by ethnicity and geography.

Demand for lactose-reduced and lactose-free dairy products has consequently surged globally. The global lactose-free dairy market was valued at USD 12.87 billion in 2024 and is projected to expand at a compound annual growth rate (CAGR) of 8.6%, reaching USD 13.98 billion in 2026 and an estimated USD 19.37 billion by 2029 (The Business Research Company, 2025) ^[17]. This growth is driven by rising health consciousness, increased diagnosis of lactose intolerance, product innovation, and expansion of retail and e-commerce channels.

Three principal industrial strategies have been deployed for lactose removal or reduction: (i) membrane-based separation (nanofiltration and chromatography), (ii) microbial fermentation

using high-lactose-fermenting strains, and (iii) enzymatic hydrolysis using lactase. Of these, enzymatic hydrolysis is the most commercially dominant because it is cost-effective, highly specific, preserves the nutritional matrix of milk, and yields a product with improved sensory properties due to the higher sweetness of glucose and galactose compared to lactose. However, free-enzyme systems are limited by poor thermal stability, sensitivity to inhibitory products (glucose and galactose feedback inhibition), non-recoverability, and potential carryover into the final product where continued enzymatic activity may impair shelf life (Deng *et al.*, 2024) [6].

Immobilization, the restriction of enzyme molecules to a solid support or within a matrix while retaining catalytic functionality overcomes these limitations by enabling enzyme reuse, enhancing operational stability, and facilitating integration into continuous flow reactors. Since the first commercial immobilized lactase systems were deployed in the 1980s, significant advances in support materials, immobilization chemistry, and reactor design have transformed the technology. This review synthesizes current knowledge on immobilized lactase systems for lactose-free dairy production, with emphasis on recent advances in nanomaterial supports, multi-enzyme systems, and process optimization.

2. Lactase (β -Galactosidase): Biochemistry and Industrial Sources

2.1 Catalytic Mechanism

Lactase catalyzes the hydrolysis of lactose according to the reaction:



The enzyme operates via a double-displacement (retaining) mechanism involving two catalytic glutamate residues. Nucleophilic attack by the first glutamate on the anomeric carbon of the galactose moiety forms a covalent galactosyl-enzyme intermediate, releasing glucose. Hydrolysis by water (or transglycosylation with lactose) then regenerates the free enzyme and yields galactose (or galactooligosaccharides, GOSs). The relative rates of hydrolysis and transglycosylation depend on lactose concentration, temperature, and pH, and are exploited industrially for GOS production, prebiotic oligosaccharides increasingly valued for gut health (Chalella Mazzocato & Jacquier, 2024) [5].

2.2 Industrial Sources

Commercial lactase preparations are predominantly of microbial origin. Three sources dominate industrial application: *Kluyveromyces lactis* (optimal pH ~7.0; suitable for neutral milk pH), *Kluyveromyces marxianus* (thermotolerant; active at 40-50 °C), and *Aspergillus oryzae/Aspergillus niger* (optimal pH 4.0-5.0; better suited to acidic whey systems). Each source confers distinct biochemical properties relevant to the dairy substrate. Bacterial sources such as *Bacillus* spp. and *Streptococcus* spp. and recombinant cold-active lactases have also been explored, particularly for cold-pasteurized milk processing where low-temperature activity minimizes undesirable side reactions (Zhou *et al.*, 2024) [20].

Key parameters governing lactase selection for immobilization include optimal temperature and pH, inhibition by product monosaccharides, molecular mass (which affects diffusion within immobilization matrices),

and glycosylation pattern (which influences surface chemistry and immobilization efficiency). Genetic engineering approaches, including rational mutagenesis and directed evolution, are increasingly deployed to customize lactases for improved thermal stability, reduced product inhibition, and enhanced compatibility with specific immobilization chemistries.

3. Principles and Methods of Lactase Immobilization

3.1 Rationale for Immobilization

Enzyme immobilization is defined as the confinement of enzyme molecules to or within a support phase, restricting their free movement while retaining catalytic activity accessible to substrates in the continuous phase. For industrial lactase applications, the primary drivers for immobilization are:

- Enzyme reusability across multiple operational cycles, reducing per-unit enzyme cost by 40-60%
- Improved thermal and pH stability due to conformational rigidity conferred by multi-point attachment
- Easy removal of the biocatalyst from the product, eliminating enzyme carry-over concerns
- Compatibility with continuous flow reactor configurations enabling high-throughput processing
- Potential for simultaneous lactose hydrolysis and GOS synthesis in a single reactor (Silva Lopes *et al.*, 2025) [14].

The optimal immobilization strategy for a given application must balance activity retention (activity of immobilized enzyme relative to free enzyme), binding strength (resistance to leakage), operational stability (activity half-life under process conditions), and practical considerations including cost, food-grade status of the support, and scale-up feasibility.

3.2 Immobilization Techniques

3.2.1 Adsorption

Physical adsorption involves attachment of lactase to a support surface through non-covalent interactions-van der Waals forces, electrostatic interactions, and hydrogen bonding. It is the simplest technique, requiring mild conditions and causing minimal enzyme denaturation. However, the weak binding renders the immobilized enzyme susceptible to leakage, particularly under high ionic strength, extreme pH, or continuous flow conditions. Common adsorbents include anion exchange resins (DEAE-cellulose), polyaniline (PANI), and ion exchange supports. PANI-immobilized lactase demonstrated 98% residual activity after 10 recycles and 100% lactose conversion after one hour (Alzahrani *et al.*, 2024) [2].

3.2.2 Covalent Binding

Covalent attachment involves the formation of stable covalent bonds between amino acid residues on the enzyme surface (typically lysine ϵ -amino groups, cysteine thiols, or carboxyl groups) and reactive functional groups on the support (epoxides, aldehydes, carbodiimides, isocyanates). Glutaraldehyde is a widely used bifunctional cross-linker that bridges enzyme amino groups to support amino groups. Eupergit C (epoxide-activated polyacrylamide beads) has been a commercial standard for dairy lactase immobilization. Covalent binding minimizes enzyme

leakage and confers high operational stability, though partial activity loss (10-40%) is common due to active site involvement or conformational restriction. Multi-point covalent attachment substantially enhances thermal stability by reducing conformational flexibility (Alzahrani *et al.*, 2024) [2].

3.2.3 Entrapment

Entrapment involves physically enclosing lactase molecules within a polymeric matrix (hydrogel) or porous structure. Calcium alginate, carrageenan, polyacrylamide, and polyvinyl alcohol are the most studied matrices. The enzyme is not chemically modified, preserving activity, but diffusion of substrate to and from the enzyme is restricted, creating mass transfer limitations particularly for large substrates. Alginate bead entrapment is inexpensive and food-grade, making it attractive for scaled applications. Diffusion can be mitigated by using thin matrix films, optimizing bead size, and employing macroporous supports.

3.2.4 Encapsulation

Encapsulation confines lactase within a semi-permeable membrane (microcapsule or hollow fibre), allowing substrates and products to diffuse through while retaining

the enzyme. Microencapsulation protects the enzyme against harsh environmental conditions and immune inactivation in oral delivery applications. In dairy processing, membrane reactors employing hollow fibres with immobilized lactase enable continuous hydrolysis of milk permeate streams. High production cost and membrane fouling remain primary challenges.

3.2.5 Cross-Linking (CLEAs)

Cross-linked enzyme aggregates (CLEAs) are prepared by precipitating the enzyme from solution (using ammonium sulfate, acetone, or ethanol) followed by cross-linking with bifunctional reagents (typically glutaraldehyde), producing carrier-free, highly dense enzyme aggregates. CLEAs exhibit high enzyme loading per unit volume and eliminate the cost of support material. However, they can be mechanically fragile and difficult to recover from reactors. Composite CLEAs incorporating magnetic nanoparticles (combi-CLEAs) address recovery issues by enabling magnetic separation. A magnetic CLEA of β -galactosidase demonstrated preservation of 80% activity after 10 reaction cycles with complete magnetic separation (Duan *et al.*, 2022) [7].

Table 1: Comparative Overview of Lactase Immobilization Methods

Method	Bond Type	Advantages	Limitations	Key Support Examples
Adsorption	Non-covalent (van der Waals, ionic, H-bond)	Simple; mild conditions; low cost; minimal activity loss	Enzyme leakage; weak binding; sensitive to pH/salt	Silica, alumina, DEAE-cellulose, ion exchange resins
Covalent Binding	Covalent (functional group)	Strong attachment; low leakage; high operational stability	Complex chemistry; partial activity loss; difficult regeneration	Eupergit C, epoxy resins, amino-silica, glutaraldehyde-activated supports
Entrapment	Physical containment	Good protection; versatile; applicable to large enzymes	Diffusion barriers; substrate size limitation; leakage of small enzymes	Alginate, polyacrylamide, carrageenan, polyvinyl alcohol
Encapsulation	Semi-permeable membrane	High protection; controlled release; good for continuous systems	High cost; membrane fouling; scale-up challenges	Liposomes, polyamide microcapsules, hollow fibers
Cross-Linking (CLEAs)	Covalent intermolecular	No carrier needed; high enzyme loading; high activity retention	Brittle structure; difficult to regenerate; activity loss during cross-linking	Glutaraldehyde (bifunctional), dextran polyaldehyde

4. Support Materials for Lactase Immobilization

4.1 Natural Polymers

Natural biopolymers, particularly alginate, chitosan, agarose, and cellulose, are favoured in food industry applications due to their generally recognized as safe (GRAS) status, biocompatibility, biodegradability, and low cost. Calcium alginate gels are among the most widely studied entrapment matrices for lactase and are used commercially in GRAS-approved systems. Chitosan, derived from chitin, provides reactive amino and hydroxyl groups enabling facile covalent functionalization and serves as an excellent coating for magnetic nanoparticles. Agarose-based supports exhibit low non-specific protein adsorption and excellent flow properties in packed-bed reactors.

4.2 Synthetic Polymers

Eupergit C (glycidyl methacrylate-co-allyl glycidyl ether copolymer beads with epoxide groups) represents the gold standard for covalent lactase immobilization in industrial dairy plants, offering high mechanical stability, defined particle size, and tunable pore structure. Polyacrylamide gels are widely used for entrapment, while polyvinyl alcohol (PVA) cryogels provide elastic, mechanically robust matrices suitable for continuous reactor operation. Synthetic

polymer supports generally offer superior mechanical stability compared to natural polymers but may lack food-grade status.

4.3 Inorganic Supports

Silica-based materials, including mesoporous silica nanoparticles (MSNs) with ordered pore architecture (MCM-41, SBA-15), offer high surface area (up to 1,000 m²/g), tunable pore size (2-50 nm), and excellent thermal and chemical stability. Lactase immobilized on MSNs has demonstrated 3-fold improvements in thermal stability relative to free enzyme. Glass microspheres as covalent carriers achieved 83.2% immobilization yield with operational reusability for more than 10 cycles and activity retention over 62 days, outperforming PAN beads, cellulose, and nylon pellets under equivalent conditions (Li *et al.*, 2018) [11]. Titanium dioxide nanoparticles have also been explored for lactase immobilization due to their photocatalytic properties and OH-rich surface.

4.4 Nanomaterials: The Next Generation

Nanomaterial-based supports have attracted intense research interest owing to their exceptionally high surface area-to-volume ratios, tunable surface chemistry, and functional

properties not available in macroscopic supports. Key nanomaterial categories include:

4.4.1 Magnetic iron oxide nanoparticles (Fe₃O₄ MNPs): Fe₃O₄ MNPs functionalized with chitosan, amino groups, or polymer coatings enable covalent lactase immobilization and magnetic recovery from reaction media, eliminating the need for centrifugation or filtration. Recombinant β-galactosidase immobilized on histidine-nickel-functionalized MNPs exhibited approximately 50-fold improvement in thermal stability relative to the free enzyme (Gennari *et al.*, 2024) ^[8]. Chitosan-coated Fe₃O₄ nanocomposites immobilized with *Aspergillus oryzae* β-galactosidase showed 15.5% GOS yield with superior recyclability (Ansari & Damanhory, 2023) ^[3].

4.4.2 Electrospun nanofibers: Electrospinning generates fibrous polymer mats with high porosity (>80%), extremely large surface areas, and tunable fiber diameter (50 nm-10 μm). Lactase immobilized on electrospun poly (acrylonitrile) or poly (vinyl alcohol) nanofibers has demonstrated enhanced pH and temperature stability relative to free enzyme. A recent study by Stoilova *et al.* (2025) ^[15] reported a multi-enzyme system combining lactase with glucose oxidase and catalase on polymer nanofibers, which reduced glucose feedback inhibition and showed prolonged

hydrolytic activity. The authors propose a "tea-bag" format for convenient low-cost lactose-free milk production, a concept with strong commercial potential.

4.4.3 Graphene oxide (GO) and carbon nanotubes: GO provides rich surface functionality (epoxide, carboxyl, and hydroxyl groups) for covalent lactase attachment, while maintaining a two-dimensional support structure with superior electrical and mechanical properties. Multi-walled carbon nanotubes (MWCNTs) have been used for *K. lactis* β-galactosidase immobilization with enhanced stability and reusability (Ansari *et al.*, 2013) ^[4]. Regulatory clearance for food contact remains an ongoing challenge for these materials.

4.4.4 Metal-organic frameworks (MOFs): Porous crystalline materials composed of metal ions coordinated to organic ligands offer ultra-high surface areas, tunable pore chemistry, and unique adsorption properties. A nickel organic framework with amorphous-crystalline phase transition properties was recently employed for efficient β-galactosidase immobilization and magnetic recycling, demonstrating high GOS synthesis capacity (Addai *et al.*, 2024) ^[1]. MOF-based enzyme supports represent a frontier area with potential for applications requiring precise control of the enzymatic microenvironment.

Table 2: Support Materials for Lactase Immobilization: Properties and Performance

Category	Support Material	Key Property	Reported Performance/Reference
Natural Polymer	Sodium alginate	Biocompatible; food-grade; easy gelation	Retained ~82% activity after 10 cycles; widely used in batch systems
Natural Polymer	Chitosan	Abundant; antimicrobial; easy functionalization	Fe ₃ O ₄ -chitosan nanocomposites showed 15.5% GOS yield; stable for 30 days (Ansari <i>et al.</i> , 2023) ^[3]
Natural Polymer	Agarose	Low non-specific adsorption; heat-stable	Used in Lactozym commercial systems; excellent flow properties
Synthetic Polymer	Eupergit C	Epoxide groups; easy covalent binding	High operational stability; used in industrial dairy plants for >100 batch reuses
Synthetic Polymer	Polyvinyl alcohol (PVA)	Elastic; easy entrapment	Suitable for continuous reactors; activity retention >75% over 15 days
Inorganic	Silica (mesoporous)	High surface area; thermally stable; tunable pore size	Improved thermal stability of lactase by ~3-fold vs. free enzyme
Inorganic	Glass microspheres	Rigid; inert; recyclable	Covalent lactase on glass: 83.2% immobilization yield; active for 62 days (Li <i>et al.</i> , 2018) ^[11]
Nanomaterial	Fe ₃ O ₄ magnetic nanoparticles	Magnetic recovery; large surface area	Recombinant β-Gal: ~50× improved thermal stability; easy magnetic separation (Gennari <i>et al.</i> , 2024) ^[8]
Nanomaterial	Graphene oxide	Large surface area; functional groups; mechanical strength	Enhanced lactase loading and catalytic efficiency; emerging food-grade applications
Nanomaterial	Electrospun nanofibers	High porosity; large area; flexible design	Multi-enzyme system (lactase + glucose oxidase): prolonged activity; "tea-bag" format potential (Stoilova <i>et al.</i> , 2025) ^[15]

5. Industrial Applications in Lactose-Free Dairy Product Manufacturing

5.1 Lactose-Free Fluid Milk

The most significant commercial application of immobilized lactase is continuous lactose hydrolysis in fluid milk processing. In packed-bed reactors, milk is pumped through columns packed with immobilized lactase at controlled temperature (typically 5-10 °C for cold processing or 35-40 °C for higher-efficiency warm processing) and flow rate, achieving lactose reduction to below 0.1 g/100 mL, the threshold for lactose-free labelling in most regulatory frameworks. Absence of enzyme in the final product prevents post-processing sweetening, off-flavour development, and Maillard browning during heat treatment.

Operational reusability exceeding 30 cycles has been reported, reducing enzyme costs by approximately 40% compared to batch free-enzyme processes (Deng *et al.*, 2024) ^[6]. Fluidized-bed reactors and magnetic-enzyme configurations represent important advances. Gennari *et al.* (2024) ^[8] demonstrated lactose hydrolysis in both packed-bed and fluidized-bed reactors using a recombinant β-galactosidase immobilized on magnetic core-shell capsules, achieving efficient lactose conversion in skim milk and cheese whey with exceptional operational stability. The magnetic support allowed straightforward enzyme recovery and recycling, addressing a key economic bottleneck of continuous processing.

5.2 Yogurt and Fermented Dairy Products

Pre-treatment of milk with immobilized lactase before fermentation reduces the initial lactose load, resulting in products with improved digestibility for lactose-intolerant consumers without compromising the fermentation process. Lower residual lactose at the end of fermentation reduces post-acidification during storage, improving product shelf life. Improved substrate availability for lactic acid bacteria due to glucose and galactose availability can accelerate fermentation kinetics, reducing manufacturing time by up to 25%. Enhanced sweetness of the fermented product may allow reduction in added sugar, aligning with clean-label formulation trends.

5.3 Cheese Production

In cheese manufacturing, lactase treatment of cheese milk or whey reduces residual lactose that would otherwise cause undesirable browning (Maillard reactions) during ripening or heat processing. Lactose reduction also benefits the flavour profile of aged cheeses, where incomplete lactose fermentation produces off-flavors. For certain soft cheeses intended for lactose-intolerant consumers, immobilized lactase allows targeted lactose reduction without altering the proteolytic or lipolytic processes central to flavor development. Lactase-treated cheese milk yields products suitable for the rapidly growing lactose-sensitive consumer segment without necessitating reformulation of established cheese recipes.

5.4 Ice Cream and Frozen Desserts

Lactose crystallization in ice cream, manifested as the sandy or gritty texture defect is a significant quality and consumer acceptance issue, particularly in high-solids formulations. Enzymatic hydrolysis of lactose prior to freezing converts it to glucose and galactose, which are more soluble and do not crystallize under typical frozen storage conditions. Immobilized lactase treatment of the ice cream mix before freezing effectively eliminates the lactose crystallization

problem while enhancing perceived sweetness enabling partial reduction of added sucrose and aligning with sugar reduction targets. Improved overrun properties have also been reported, contributing to a lighter texture (Vallejo Garcia *et al.*, 2024) ^[18].

5.5 Infant Formula

Low-lactose and lactose-free infant formulas are essential for infants with congenital lactase deficiency or secondary lactase insufficiency following gastrointestinal disease. Immobilized lactase technology offers controlled lactose reduction while preserving the nutritional integrity of the base milk in particular, the heat-sensitive whey proteins and bioactive components that are susceptible to degradation during prolonged heat treatment. Regulatory requirements for infant formula production demand stringent enzyme-free final products, making immobilized systems which are removed prior to finishing inherently superior to free-enzyme batch processes.

5.6 Whey Processing and Galactooligosaccharide Production

Dairy whey generated in large volumes during cheese and casein manufacture contains 4-5% lactose and represents a significant effluent management challenge as well as a valorization opportunity. Immobilized β -galactosidase in packed-bed or membrane reactors enables continuous lactose hydrolysis of whey permeate, reducing biological oxygen demand and producing glucose-galactose syrups useful as fermentation substrates or sweeteners. At elevated lactose concentrations, the transglycosylation activity of β -galactosidase can be harnessed to synthesize galactooligosaccharides (GOSs), a bifidogenic prebiotic oligosaccharides with demonstrated benefits for gut microbiome composition and immune function. Fe₃O₄-chitosan-immobilized β -galactosidase achieved GOS yields of up to 15.5% (w/v) with excellent recyclability in batch systems (Ansari & Damanhory, 2023) ^[3].

Table 3: Immobilized Lactase Systems in Dairy Product Manufacturing: Applications and Outcomes

Product	Reactor Type	Key Benefits	Technical Outcome
Lactose-free milk	Packed-bed/Fluidized-bed continuous reactor	Continuous operation; no enzyme in final product; high productivity	Complete lactose hydrolysis (<0.1 g/100 mL); reuse >30 cycles; reduced enzyme costs by ~40%
Lactose-free yogurt	Batch immobilized bioreactor (pre-fermentation)	Better digestibility; faster fermentation; improved texture and sweetness	Reduced fermentation time by up to 25%; improved consumer acceptability
Lactose-free cheese	Batch or semi-continuous treatment	Residual lactose <0.1%; improved flavor development	Enhanced suitability for lactose-intolerant consumers; reduced bitterness
Lactose-free ice cream	Batch pre-treatment	Prevents lactose crystallization; smoother texture; enhanced sweetness	No sandiness defect; improved overrun; reduced sucrose needed
Low-lactose infant formula	Membrane reactor/batch immobilized system	Preserved nutritional quality; precise lactose reduction	Lactose reduced to <0.5 g/100 mL while retaining proteins and vitamins
Whey processing	Packed-bed reactor with magnetic enzyme	Valorization of whey; GOS synthesis; reduced lactose in permeate	GOS yield up to 15.5%; magnetic separation enables easy enzyme recovery (Gennari <i>et al.</i> , 2024) ^[8]

6. Recent Technological Advances

6.1 Nanotechnology-Enabled Immobilization

The convergence of nanotechnology with enzyme engineering has opened new avenues for lactase immobilization with performance characteristics unattainable with macroscopic supports. Nanomaterial-based supports offer surface areas of 100-1,000 m²/g, enabling high enzyme loading; reduced mass transfer limitations due to nanoscale particle size and unique functional properties (magnetism, photocatalysis, tunable surface chemistry). Multiple studies have demonstrated that

nanostructured supports improve kinetic parameters notably, an increase in apparent V_{max} and decrease in apparent K_m for lactose compared to free enzyme under equivalent conditions reflecting both increased enzymes loading and facilitated substrate accessibility (Zhou *et al.*, 2024) ^[20].

6.2 Multi-Enzyme Cascade Systems

An emerging strategy is the co-immobilization of lactase with auxiliary enzymes on the same support to address cascade reactions or feedback inhibition. A critical limitation of lactase-catalyzed lactose hydrolysis is glucose

feedback inhibition, as glucose accumulates, it competitively inhibits lactase, reducing the rate of hydrolysis. Stoilova *et al.* (2025) ^[15] addressed this by co-immobilizing lactase with glucose oxidase (GOx) and catalase on polymer nanofibers. GOx oxidizes glucose to gluconic acid, reducing the inhibitory glucose concentration and simultaneously generating H₂O₂, which is then detoxified by catalase. The multi-enzyme system-maintained lactose hydrolysis activity substantially longer than single-enzyme systems, representing a significant advance in biocatalyst efficiency for continuous dairy processing.

6.3 Oriented Immobilization

Conventional immobilization relies on random attachment of the enzyme to the support, which may involve amino acid residues in or near the active site, causing partial or complete loss of catalytic function. Oriented immobilization which directs enzyme attachment away from the active site maximizes activity retention. His tag based oriented immobilization, exploiting specific coordination of the polyhistidine sequence with Ni²⁺-functionalized supports, allows recombinant lactases to be immobilized in a defined orientation. Gennari *et al.* (2024) ^[8] demonstrated that His-tagged recombinant β -galactosidase on Ni²⁺-functionalized magnetic nanoparticles showed increased substrate affinity and approximately 50-fold improved thermal stability compared to the free enzyme, with excellent operational performance for lactose hydrolysis in skim milk and cheese whey.

6.4 Food-Grade and Sustainable Immobilization Systems

Industrial deployment of immobilized lactase in food systems requires that support materials meet food safety standards, particularly GRAS classification under FDA regulations or equivalent international frameworks. Recent research has focused on developing food-grade immobilization matrices that are safe for incidental food contact, including chitosan, food-grade alginate, carrageenan, gellan gum, and cellulose-based materials. Chalella Mazzocato and Jacquier (2024) ^[5] provided a comprehensive review of recent advances in food-grade immobilization systems, highlighting that safety, stability, and scalability must be simultaneously addressed for regulatory approval. Sponge-based materials are inexpensive, porous, and mechanically robust have recently been explored as novel supports for β -galactosidase cross-linked enzyme aggregates, offering high surface area and mass transfer efficiency at low cost (LWT, 2024) ^[12].

6.5 Continuous Bioreactor Configurations

Packed-bed reactors (PBRs), fluidized-bed reactors (FBRs), and membrane bioreactors (MBRs) are the principal continuous configurations employed for immobilized lactase in dairy processing. PBRs offer high enzyme density and simple operation but are prone to fouling and pressure drop build-up with fat-containing dairy streams; they perform best with clarified whey permeate or skim milk. FBRs provide better mass transfer through fluid-particle contact and are more tolerant of particulate feeds. MBRs particularly hollow fibre configurations decouple enzyme retention from substrate flow, enabling simultaneous hydrolysis and product removal. Spiral-wound membrane bioreactors have been used for whey lactose hydrolysis with

extended operational periods and consistent productivity (Vasileva *et al.*, 2016) ^[19]. Recent computational approaches using computational fluid dynamics (CFD) modeling are improving reactor design by optimizing flow distribution and minimizing enzyme denaturation zones.

6.6 Artificial Intelligence and Process Optimization

Artificial intelligence (AI) and machine learning (ML) tools are beginning to transform immobilized enzyme process development. AI algorithms can predict optimal immobilization conditions (pH, temperature, cross-linker concentration, support chemistry) from prior experimental data, substantially reducing the experimental screening burden. In reactor operation, ML-based process control systems monitor substrate conversion in real time and dynamically adjust operating parameters like flow rate, temperature, pH to maintain target lactose hydrolysis levels while maximizing enzyme longevity. Protein engineering guided by ML is enabling the rational design of thermostable, inhibitor-tolerant lactases optimized for specific immobilization platforms, accelerating the development of next-generation biocatalysts (IntechOpen, 2025) ^[9]. These advances collectively indicate that AI-integrated immobilized enzyme systems represent the future of industrial lactose-free dairy production.

7. Challenges and Limitations

Despite significant progress across immobilization chemistry, support materials, and reactor design, several technical, economic, and regulatory challenges continue to impede the full potential of immobilized lactase technology at industrial scale.

Mass transfer limitations remain the most fundamental technical constraint of immobilized enzyme systems. When lactase is entrapped within a polymeric matrix or confined within a porous support, diffusion of the large lactose molecule (MW 342 Da) to the enzyme active site may be rate-limiting, reducing the apparent catalytic rate. This limitation is particularly pronounced in alginate beads of conventional size (1-3 mm), and necessitates miniaturization of the matrix (nanoparticles, thin films, nanofibers) or adoption of open-pore architectures to restore adequate substrate accessibility.

Activity loss during immobilization is inherent to most covalent and cross-linking strategies. When reactive groups are present near the active site of lactase, their modification during immobilization directly reduces catalytic capacity. Multi-point attachment enhances stability but amplifies this effect. Optimizing the orientation of immobilization through his-tag systems or site-directed chemical modification can minimize this problem but adds process complexity and cost.

Economic viability at industrial scale remains a significant barrier, particularly for nanostructured and specialty supports. Graphene oxide, electrospun nanofibers, and MOF-based supports offer impressive performance in laboratory studies but their manufacturing costs are substantially higher than conventional Eupergit C or alginate systems. Demonstrating sufficient enzyme loading, reuse cycles, and operational stability to justify the premium cost is essential for commercial adoption.

Regulatory compliance, particularly with regard to the use of nanomaterials in food contact applications, is an evolving challenge. Most regulatory frameworks have not yet

established clear approval pathways for engineered nanomaterials in direct food processing applications, creating uncertainty for industrial developers. The European Food Safety Authority (EFSA) and the United States FDA are developing guidance frameworks, but approvals remain limited. This regulatory gap constrains the commercial deployment of the most technologically advanced immobilized systems despite their superior performance characteristics (Chalella Mazzocato & Jacquier, 2024) [5].

Scale-up from laboratory to pilot to industrial scale frequently reveals engineering challenges including pressure drop, channeling, fouling by milk fat and proteins, microbial contamination, and inadequate heat management—that are not apparent at bench scale. Dairy streams containing fat and casein micelles are particularly challenging substrates for packed-bed systems, often requiring pre-clarification or membrane pre-filtration steps that add process complexity and cost.

Table 4: Key Challenges in Immobilized Lactase Technology and Future Mitigation Strategies

Challenge Area	Current Status	Future Direction/Mitigation Strategy
Enzyme leakage (adsorption)	Significant leakage under high ionic strength or pH shifts; reduces reusability	Combine with covalent binding or cross-linking; use functionalized supports with strong affinity groups
Mass transfer limitations	Diffusion barriers in entrapment matrices reduce effective substrate accessibility	Nanoporous supports; electrospun nanofibers; hollow fiber membrane reactors with thin matrices
Activity loss on immobilization	10-40% activity loss common after chemical immobilization steps	Oriented immobilization via His-tag or biotin-avidin; mild cross-linkers; directed evolution of lactase
High immobilization cost	Specialty supports (graphene, MSNs, nanofibers) remain expensive	Food-grade low-cost supports (alginate, chitosan, sponge); scale-up optimization; enzyme recycling protocols
Scale-up difficulties	Lab-scale results often not replicated at industrial scale due to flow, fouling, and pressure issues	Modular packed-bed and fluidized-bed reactor designs; AI-assisted process control and optimization
Regulatory compliance	Food-grade status not established for most nanocarriers; consumer safety data lacking	Focus on GRAS-approved supports; rigorous safety evaluation; transparency in labeling
Support material lifespan	Most supports degrade after 10-30 cycles or 30-60 days of continuous use	3D-printed durable reactors; magnetic nanoparticle-based systems enabling rapid external recovery

8. Future Perspectives

The trajectory of immobilized lactase research points toward several convergent developments that are likely to define the next generation of lactose-free dairy processing technology. Smart and responsive immobilization materials supports that can modulate enzyme activity in response to external stimuli (temperature, pH, magnetic field, or light) represent an exciting frontier. Thermoresponsive polymer coatings (e.g., poly-N-isopropylacrylamide) can be engineered to alter porosity and substrate accessibility in response to temperature, enabling "on-demand" enzyme activation and inhibition that could be coupled with temperature cycling in process control systems.

Magnetic enzyme recovery systems, incorporating superparamagnetic nanoparticle cores, are expected to become increasingly commercially viable as scalable nanoparticle synthesis methods reduce costs. These systems eliminate the need for centrifugation or filtration and enable continuous enzyme recycling in stirred-tank reactors, a configuration more amenable to whole milk processing than packed-bed systems (Gennari *et al.*, 2024) [8].

Three-dimensional (3D) printing of enzyme reactor components using food-compatible materials opens opportunities for the fabrication of geometrically optimized reactor structures including customized flow channels, integrated heat exchangers, and enzyme-loaded structured packings that are unattainable through conventional manufacturing. 3D-printed alginate-lactase constructs have been demonstrated at laboratory scale, and scale-up is anticipated as 3D bioprinting technology matures.

AI-assisted directed evolution of lactase is expected to accelerate the development of enzyme variants with dramatically improved stability, reduced product inhibition, and optimized compatibility with specific immobilization chemistries. By training ML models on sequence-function-stability datasets from large enzyme libraries, it is possible to predict beneficial mutations without exhaustive experimental screening. Such customized lactase variants

could be engineered in tandem with their intended support material, creating a co-designed enzyme-support system optimized from the ground up for industrial dairy processing conditions.

Integrated biotransformation platforms combining lactose hydrolysis with GOS synthesis, milk sugar isomerization (lactulose production), or valorization of hydrolysis products into high-value metabolites within a single immobilized multi-enzyme reactor represent the long-term vision for sustainable dairy bioprocessing converting a potential waste stream (lactose) into multiple value-added products simultaneously.

9. Conclusion

Immobilized lactase technology has matured from a laboratory concept to a cornerstone of industrial lactose-free dairy production, driven by the global imperative to address the digestive needs of the estimated 68-70% of adults worldwide affected by lactose malabsorption. By enabling enzyme reuse, improving operational stability, eliminating enzyme carry-over into products, and facilitating continuous manufacturing, immobilized lactase systems offer compelling economic and technical advantages over free-enzyme processes across the full spectrum of dairy applications from fluid milk and yogurt to cheese, ice cream, infant formula, and whey valorization.

Advances in nanomaterial-based supports, including magnetic nanoparticles, electrospun nanofibers, and MOF-based carriers, are redefining the performance benchmarks for enzyme stability, loading, and recovery. Multi-enzyme cascade systems that address the glucose feedback inhibition problem, oriented immobilization strategies that maximize activity retention, and AI-guided process optimization are collectively advancing the technology toward its full industrial potential.

Significant challenges remain principally in mass transfer efficiency, scale-up engineering, economic viability of advanced supports, and regulatory pathways for

nanomaterials in food contact applications. Addressing these challenges through interdisciplinary collaboration between food technologists, biochemical engineers, material scientists, and regulatory scientists will be essential to realize the full potential of immobilized lactase as a sustainable, high-performance biocatalytic platform for the growing global lactose-free dairy sector.

10. Declarations

10.1 Ethical approval

Ethical approval was not required for this review article because no animal experimentation, human participation, or collection of primary biological samples was involved.

10.2 Conflict of interest

The authors declare that they have no conflict of interest.

10.3 Funding

The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors for the preparation of this review article.

10.4 Data availability statement

No new data were generated or analyzed in this study. Data sharing does not apply to this article.

10.5 AI disclosure statement

The authors declare that artificial intelligence (AI) tools were used only for language refinement/grammar assistance where applicable. All scientific interpretation, literature synthesis, critical analysis, and final manuscript preparation were performed by the authors.

References

1. Addai FP, Wu J, Liu Y, Ma X, Han J, Lin F, *et al.* Amorphous-crystalline phase transition and intrinsic magnetic property of nickel organic framework for easy immobilization and recycling of β -galactosidase. *Int J Biol Macromol.* 2024;254:127901.
2. Alzahrani F, Akanbi TO, Scarlett CJ, Aryee AN. The use of immobilised enzymes for lipid and dairy processing and their waste products: A review of current progress. *Processes.* 2024;12(4):634.
3. Ansari SA, Damanhory AA. Biotechnological application of *Aspergillus oryzae* β -galactosidase immobilized on glutaraldehyde modified zinc oxide nanoparticles. *Heliyon.* 2023;9:e13089.
4. Ansari SA, Satar R, Chibber S, Khan MJ. Enhanced stability of *Kluyveromyces lactis* β -galactosidase immobilized on glutaraldehyde modified multiwalled carbon nanotubes. *J Mol Catal B Enzym.* 2013;97:258-263.
5. Chalella Mazzocato M, Jacquier JC. Recent advances and perspectives on food-grade immobilisation systems for enzymes. *Foods.* 2024;13(13):2127.
6. Deng Z, Deng Q, Li B, Li J, Jung S, Cho NJ, *et al.* Strategies for lactase immobilization and delivery to relieve lactose intolerance. *Trends Food Sci Technol.* 2024;143:104244.
7. Duan F, Sun T, Zhang J, Wang K, Wen Y, Lu L. Recent innovations in immobilization of β -galactosidases for industrial and therapeutic applications. *Biotechnol Adv.* 2022;61:108053.
8. Gennari A, Simon R, Renard G, *et al.* Lactose hydrolysis in packed-and fluidized-bed reactors using a recombinant β -galactosidase immobilized on magnetic core-shell capsules. *Bioprocess Biosyst Eng.* 2024;47:263-273.
9. IntechOpen. Optimizing enzymes for dairy processing: A protein engineering perspective. 2025.
10. Li A, Zheng J, Han X, Yang S, Cheng S, Zhao J, *et al.* Advances in low-lactose/lactose-free dairy products and their production. *Foods.* 2023;12(13):2553.
11. Li Z, Shi H, Yang Y, Liang H. Lactose-free milk preparation by immobilized lactase in glass microsphere bed reactor. *Food Biophys.* 2018;13:216-224.
12. Immobilization of β -galactosidase within sponge: Hydrolysis of lactose in milk. *LWT Food Sci Technol.* 2024;215:115779.
13. Robescu MS, Bavaro T. A comprehensive guide to enzyme immobilization: All you need to know. *Molecules.* 2025;30(4):939.
14. Silva Lopes PH, Nelson DL, Damasceno SM. Enzyme immobilization: Advancements, techniques, and industrial applications. *Curr Enzyme Inhib.* 2025.
15. Stoilova O, Ignatova M, Manolova N. Immobilization of enzymes for synergy in polymers to produce lactose free milk. *Sci Rep.* 2025;15:17402.
16. Storhaug CL, Fosse SK, Fadnes LT. Country, regional, and global estimates for lactose malabsorption in adults: A systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2017;2(10):738-746.
17. The Business Research Company. Lactose-free dairy global market report. 2025.
18. Vallejo García JLAU, Cutillo Foraster A, Arnáiz Alonso AAU, Vallejos Calzada SAU, García Pérez JMAU, Muñoz Santamaría MAA, *et al.* Hydrolysis of lactose: Conventional techniques and enzyme immobilization strategies on polymeric supports. In: *Milk Proteins—Technological Innovations, Nutrition, Sustainability and Novel Applications.* 2024. p. 45-89.
19. Vasileva N, Ivanov Y, Damyanova S, Kostova I, Godjevargova T. Hydrolysis of whey lactose by immobilized β -galactosidase in a bioreactor with a spirally wound membrane. *Int J Biol Macromol.* 2016;82:339-346.
20. Zhou M, Chen X, Zheng J, Li S. β -Galactosidase: Insights into source variability, genetic engineering, immobilisation and diverse applications in food, industry and medicine. *Int J Dairy Technol.* 2024;77(4):812-835.