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Enzymatic dispersal of biofilms in the food industry: mechanisms, applications, and future prospects

Muhammad Afiqamzar Azmi[1], Mohd Taufiq Mat Jalil[1], Qingyu Zhang[1,2] and Mohd Fakharul Zaman Raja Yahya[1,3*]

[1] *Faculty of Applied Science, Universiti Teknologi MARA Shah Alam, 40450 Shah Alam, Selangor, Malaysia*
[2] *Department of Food Research, Zhejiang Business College, Hangzhou, Zhejiang 310059, China*
[3] *Integrative Pharmacogenomics Institute (iPROMISE), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia*
* Correspondence: fakharulzaman@uitm.edu.my (Yahya M)

Abstract

The formation of biofilms by pathogenic and spoilage microorganisms is an ongoing challenge in the food industry, because it leads to contamination, antimicrobial resistance, and recurrent outbreaks that compromise food safety and quality. The conventional chemical and physical strategies often fail to prevent biofilm formation because of the presence of extracellular polymeric substances that protect embedded cells. This necessitates the development of innovative solutions to ensure effective hygiene in food products. Therefore, this work aims to determine the role of enzymatic dispersal as a targeted strategy in degrading biofilm matrices and enhancing microbial eradication, with an emphasis on its recent applications in the food industry. In this work, we summarize enzymatic dispersal of biofilms, discuss the underlying mechanisms of action, and evaluate its practical integration into industrial cleaning protocols. By highlighting the ability of enzymes such as glycosidases, proteases, and DNases to disrupt the structural integrity of biofilms and intensify the action of sanitizers, this work provides important insights into the potential of enzymatic approaches as sustainable, eco-friendly alternatives to the conventional methods. This ultimately improves food safety management and reduces biofilm-mediated risks in production environments. It directly addresses the major, ongoing challenge of microbial biofilms in food production, a primary source of contamination, spoilage, and foodborne illness outbreaks that have significant consequences on the public health and economy. Future work should focus on optimizing enzyme cocktails (combination of glycosidases, proteases, and DNases) designed to disrupt the biofilm matrices formed by prevalent foodborne pathogens.

Keywords: Biofilm, Enzymatic dispersal, Food industry, Enzymes, Eco-friendly

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Introduction

In the food industry, biofilms are gaining recognition as one of the most persistent challenges to food safety and quality. In food processing environments, it is common that bacterial cells tend to attach to surfaces, secrete extracellular polymeric substances (EPSs), and form structured biofilm communities that prevent conventional cleaning and disinfection. It is well known that biofilm-grown bacteria are up to 1,000-fold more resistant than planktonic cells.[1], which results in recurrent contamination issues and product recalls. The economic burden caused by biofilm contamination is significant, thereby encompassing losses from food spoilage, product recalls, reduced shelf life, and increased sanitation costs[2]. In addition, the global annual cost incurred by biofilm prevention through food packaging has been estimated at US $303 billion[3]. Traditional antimicrobial strategies, including the use of chemical disinfectants and physical treatments, often fail to fully eradicate biofilms, especially when they mature and develop protective EPS layers. In recent years, enzymatic dispersal has emerged as a promising antibiofilm strategy that could complement or replace the conventional cleaning methods in the food industry[4].

Enzymatic dispersal has been gaining interest because of its potential to target specific structural components of the biofilm matrix, degrade their protective barriers, and facilitate the penetration of the antimicrobial agents[5]. Unlike broad-spectrum disinfectants, enzymatic treatment can be customized to attack specific biofilm constituents such as polysaccharides, extracellular DNA (eDNA), or proteins, thereby destabilizing the biofilm integrity[6]. This specificity makes enzymatic dispersal less likely to induce antimicrobial resistance compared with traditional antimicrobials. Moreover, enzymes are biodegradable, can be produced sustainably, and are generally regarded as safe for food applications. At present, more than dealing with the presence of enzymes, their successful synthesis and critical evaluation of the knowledge required for the transition from a promising laboratory concept to a viable industrial solution are crucial. This review highlights the recent advances in the enzymatic antibiofilm approaches relevant to the food industry, focusing on enzyme types, mechanisms of action, delivery methods, and potential integration into food processing workflows.

Biofilm structure and the need for enzymatic dispersal

The resilience of biofilms largely stems from their complex and robust extracellular matrix (Fig. 1). It primarily comprises three principal biopolymers: polysaccharides, proteins, and eDNA, which together form a hydrated, protective network essential for the stability and function of biofilms[7–9]. Polysaccharides are responsible for the viscous and adhesive nature of the matrix, facilitating cell attachment to surfaces common in food processing environments, such as stainless steel or plastic. Proteins within the matrix play important structural roles, often including amyloid fibers like curli

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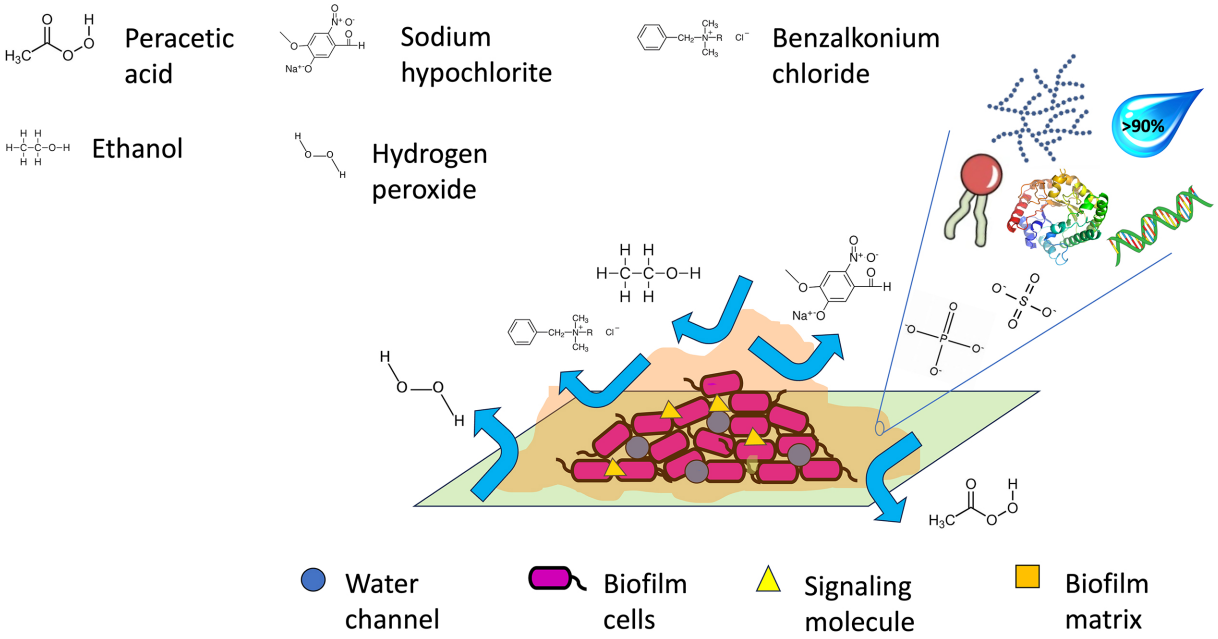

Fig. 1 Extracellular matrix provides protection against common chemical disinfectants in food processing.

found in *Escherichia coli* and *Salmonella*, which enhance surface adhesion and biofilm robustness even against proteases and harsh sanitizers. Meanwhile, eDNA aids in the initial bacterial aggregation and helps maintain the biofilm architecture. This multifaceted matrix not only stabilizes the bacterial community but also traps nutrients and protects the cells from environmental stresses, antimicrobial agents, and host immune responses[10,11], thus playing a crucial role in biofilm persistence and pathogenicity in food production settings. The conventional cleaning approaches in food plants, such as the use of chlorine-based sanitizers, quaternary ammonium compounds, and peracetic acid, may kill bacteria living on biofilm surfaces[12], but often fail to eradicate the extracellular matrix components, which remain intact and enable rapid regrowth and recolonization by bacteria. Mature biofilms are highly persistent, and they can survive repeated cleaning cycles, particularly in niches like pipelines, drains, conveyor belts, and cutting surfaces where mechanical disruption is limited[13]. This residual matrix results in the formation of a scaffold for new biofilm formation, which undermines cleaning efforts and increases contamination risk. Therefore, targeting the entire matrix directly rather than only the bacteria is increasingly recognized as essential for effective biofilm control in the food industry.

Enzymatic dispersal agents specifically address this need by particularly degrading the structural components of the extracellular matrix. Enzymes such as polysaccharidases, proteases, and DNases target polysaccharides, proteins, and eDNA, respectively, ultimately breaking them down in order to weaken the biofilm matrix[3]. Enzymatic disruption of the EPS breaks the biofilm's cohesion, resulting in the release of embedded bacterial cells into a planktonic state, where they are exposed to sanitizers and physical removal. This transition from a protected biofilm community to planktonic cells is crucial because biofilm bacteria are generally more resistant than free-floating cells. Moreover, enzymes can act synergistically with conventional disinfectants, serving as a pretreatment that enhances sanitizer penetration and overall efficacy. For example, Kuk et al.[14] reported a decrease in the number of *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* cells upon exposure to Vantocil and Catamine biocides as well as protease and amylase enzymes. The optical density of the washing solutions with biofilms decreased 4.1 times ($p < 0.05$), compared with treatment with biocides only.

The benefits of enzymatic treatment are particularly well suited for the operational reality of the food industry. Food processing equipment and surfaces contain hard-to-reach areas where cleaning-in-place (CIP) systems find it difficult to fully contact biofilms, making enzymatic degradation a valuable adjunct. According to Pant et al.[15], enzyme-based CIP offers sustainability advantages, including reduced water usage, lower operating temperatures, and biodegradable wastes. Enzymes are biodegradable, specific, and generally safe for food applications, aligning with the increasing regulatory and consumer demands for sustainable and nontoxic cleaning agents. Furthermore, designing enzymatic blends to different biofilm compositions, reflecting variability among bacterial species and environmental conditions, permits customization to diverse industrial scenarios. This adaptability helps address the inherent heterogeneity of biofilms produced by different microbial communities under varying shear forces, nutrient availability, and temperatures commonly encountered in food production. In 2012, Marcato-Romain et al.[16] demonstrated the importance of customized enzymatic approaches. They screened eight hydrolytic enzymes on a 24-h multispecies biofilm. Glycosidases and lipases were inefficient or only slightly effective at biofilm reduction. In contrast, proteases showed significantly higher efficiency. For example, a 24-h treatment with pepsin resulted in a more than 80% biofilm removal. Pepsin, the most suitable compound for industrial conditions, was tested on an industrial biofilm sample. Continued research into optimal enzyme combinations, immobilization on surfaces, and integration with cleaning workflows can revolutionize biofilm management in the food industry, shifting the paradigm from partly effective eradication to proactive and comprehensive biofilm control. The major classes of biofilm-dispersing enzymes are distinguished by the key matrix components they attack, namely, polysaccharides, eDNA, proteins, and lipids.

Enzymes targeting biofilm polysaccharides

Polysaccharides are one of the most abundant and critical components of the biofilm EPS matrix, serving as the structural scaffold that holds biofilm communities together. Different bacterial species yield structurally distinct exopolysaccharides with unique properties. For example, in *P. aeruginosa*, Polysaccharide synthesis locus (Psl) increases the matrix elasticity through cross-linking to facilitate microcolony formation, while Pellicle polysaccharide (Pel) increases the matrix viscosity to promote biofilm spreading[17]. Moreover, the polysaccharide matrix creates a hydrated, gel-like environment that not only maintains mechanical stability but also protects biofilm bacteria from desiccation and environmental stresses[18]. It facilitates adhesion to both biotic and abiotic surfaces commonly found in food processing environments, such as stainless steel, plastic, and rubber. In addition to structure, polysaccharides also help in charge shielding, thereby reducing the electrostatic repulsion between bacterial cells and surfaces to promote tighter aggregation[19].

One of the promising antibiofilm strategies involves degradation of polysaccharides by enzymes (Table 1), known as glycoside hydrolases. Dispersin B (DspB) is a particularly well-studied glycoside hydrolase enzyme with high antibiofilm activity. Isolated from *Aggregatibacter actinomycetemcomitans*, DspB specifically hydrolyzes β-1,6-linked N-acetylglucosamine polymers, key polysaccharide components responsible for the stabilization of the biofilms of pathogens such as *S. epidermidis* and *E. coli*[20]. In 2023, Breslawec et al.[21] investigated the molecular basis for the endo-glycosidic cleavage activity of DspB and its significance in the dispersal of poly-N-acetylglucosamine (PNAG)-dependent *S. epidermidis* biofilms. They revealed that anionic amino acid (D242) facilitates the endo-glycosidase activity of DspB through electrostatic interactions with cationic substrates in the −2 binding site. The application of DspB on the biofilms formed on typical food processing surfaces like stainless steel and plastic resulted in their significant reduction[22]. This enzymatic approach is especially useful in biofilms present in dairy and meat processing plants, where the aforementioned pathogens pose a persistent contamination risk through biofilm formation.

In addition to DspB, other polysaccharide-degrading enzymes are under investigation for application in the food industry. Alginate lyases are effective against alginate-rich biofilms produced by *P. aeruginosa*, a bacterium commonly found in water systems and food processing equipment. Although *P. aeruginosa* itself is not a classical foodborne pathogen, it serves as a model organism for studying biofilm dispersal mechanisms due to its robust alginate-based matrix. The antibiofilm efficacy of DspB was elucidated, for example, by Blanco-Cabra et al.[23], who demonstrated that alginate lyases with both polyM and polyG degradation activities, such as Alg2A and A1-II', effectively dissolved *P. aeruginosa* biofilms and exhibited synergistic effects with antibiotics. Dextranases and amylases were also found to be effective in degrading dextran-rich biofilms formed by spoilage bacteria such as *Leuconostoc* in sugar-rich environments[24,25]. These enzymes help disrupt biofilms that interfere with food quality and shelf life, especially in sugar-based food processing activities. This expanding enzymatic toolkit highlights the importance of targeting diverse polysaccharides that reflects the heterogeneity of biofilms present in various food processing niches.

The functionality of polysaccharides in the biofilm matrix is intricately linked to their enzymatic degradation approaches, illustrating the potential of targeting matrix components for improved biofilm control. By breaking down polysaccharide scaffolds, enzymes disrupt the physical integrity of biofilms, thereby facilitating bacterial detachment and exposing embedded cells to sanitizers and cleaning procedures[26]. This enzymatic degradation overcomes the limitations of conventional cleaning agents in penetrating or dismantling the protective matrix. In addition, enzyme treatments can be integrated into cleaning regimes to enhance overall efficacy, enabling more comprehensive biofilm removal from hard-to-reach and complex surfaces within food processing plants[27]. This strategy is gaining attention not only for its scientific merit but also for its alignment with sustainable and safe food industry practices.

Enzymes targeting the eDNA

Currently, eDNA is a well-established fundamental structural polymer within the matrix of many bacterial biofilms, far beyond its conception as merely a remnant of dead cells. It is recognized to be originated from a dual process: it can be released through programmed cell lysis, a form of autolysis that is often a controlled part of biofilm development, and also through active secretion mechanisms via membrane vesicles[28,29]. In the extracellular space, DNA strands function as a highly effective cross-linking agent, binding to other matrix components like polysaccharides, proteins, and amyloid fibers. This interaction creates a sticky, cohesive network that in turn offers mechanical stability, enhances adhesion to surfaces, and facilitates the horizontal gene transfer of traits such as antibiotic resistance, thereby further entrenching the biofilm community[30]. Tetz et al.[31] demonstrated that eDNA fragments of approximately 30 kb were always present in biofilm matrices across various Gram-positive and Gram-negative bacterial species.

Table 1. Polysaccharidases and their targets.

Enzyme class	Primary target substrate(s) in biofilm EPS	Mechanism of Action in Biofilm Dispersal
α-Amylases	Starch, glycogen, and other α-glucans (α-1,4 glycosidic bonds)	Hydrolyzes internal α-1,4 linkages in polysaccharides, disrupting carbohydrate-based matrix cohesion.
Dextranases	Dextran (α-1,6 glucan with α-1,3 branches)	Cleaves α-1,6 linkages in dextran, a key matrix polymer in many streptococcal and lactococcal biofilms.
Cellulases	Cellulose (β-1,4 glucan)	Degrades cellulose fibers by breaking β-1,4 bonds, destabilizing the structural scaffold of biofilms.
Hyaluronidases	Hyaluronic acid (β-1,4/β-1,3 glucuronate-N-acetylglucosamine)	Breaks down hyaluronan, a glycosaminoglycan that contributes to biofilm viscosity and adhesion.
Pectinases	Pectin (α-1,4 galacturonan)	Degrades pectin, a plant-derived polysaccharide that can be incorporated into biofilms in produce-handling environments.
Dispersin B	Poly-β-1,6-N-acetyl-D-glucosamine (PNAG)	Hydrolyzes the glycosidic bonds in PNAG, a major adhesin and matrix component in many bacterial biofilms.
Xylanases	Xylan (β-1,4 xylose backbone)	Degrades xylan hemicellulose, which may be present in biofilms formed in grain or plant processing facilities.

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Given its critical structural role, eDNA is a prime target for antibiofilm strategies, with DNase I emerging as the prototypical enzyme for its degradation. This enzyme catalyzes the hydrolysis of phosphodiester bonds in DNA, leading to the breakage of long, reinforcing polymers into fragments, thus causing the collapse of the biofilm's architecture. The efficacy of this approach has been demonstrated across a spectrum of significant foodborne pathogens. For instance, Lin et al.[32] showed that the inhibition and disruption of *S. aureus* biofilms by lysostaphin (Lst) were significant upon mixing with DNase I. The enzyme removed eDNA, affecting biofilm formation, dispersing mature biofilms, and thereby facilitating the penetration of Lst. By degrading eDNA, DNAse I may create pores and channels within the biofilm, thereby increasing its permeability[33]. This physical alteration facilitates better penetration of antibiotics and disinfectants, enhancing their antimicrobial efficacy against biofilm-embedded cells.

In general, the transition from a promising laboratory result to a robust, industrial-scale application involves significant practical hurdles. In this case, the primary challenges are the high cost of producing pure, food-grade DNase I and its inherent fragility under the harsh conditions typical of industrial cleaning[34]. The activity of the enzyme can be rapidly diminished by the high temperatures in hot washes, extreme pH levels from alkaline or acidic cleaners, and the presence of proteolytic enzymes in cleaning formulations or within the biofilm itself. Consequently, its stability and longevity in real-world settings remain a major impediment to widespread adoption. The current research is actively focused on overcoming the aforementioned barriers through innovative biotechnological solutions. Enzyme engineering techniques, such as directed evolution and rational protein design, are being used to produce novel DNase variants with enhanced thermostability, resistance to proteolytic degradation, and optimal activity across a wider pH range[35]. Furthermore, advances in enzyme immobilization offer a parallel pathway; by anchoring DNase molecules onto solid supports or nanoparticles, it is possible to reuse the enzyme multiple times and protect from denaturing conditions, thereby drastically improving cost-effectiveness. For example, Lin et al.[36] loaded DNase I and glucose oxidase (GOX) on chitosan nanoparticles (CSNPs) to explore their inhibitory effects on and disruption of dual-species biofilms of *Salmonella enterica* and *Listeria monocytogenes*. Compared to free DNase I or GOX, DNase I and GOX loaded on CSNPs showed higher stability at different temperatures. CSNP-DNase-GOX was more effective in inhibiting dual-species biofilms than CSNP-GOX. Together, these advancements in bioengineering pave the way for the development of next-generation, stabilized enzymatic formulations that are both economically viable and operationally practical for routine use in food safety protocols.

Enzymes targeting biofilm proteins

Proteins are a highly versatile and functional class of molecules within the EPS that forms the biofilm matrix. Their roles are multifaceted: they act as adhesins for initial surface attachment and cell–cell cohesion, as structural scaffolds that provide mechanical resilience, and as functional enzymes that aid in nutrient acquisition and community defense[37,38]. This proteinaceous framework is often cross-linked with other components such as eDNA and polysaccharides, producing a complex and robust architecture that protects embedded cells from external environment. In short, this critical structural and functional contribution makes matrix proteins a prime target for enzymatic disruption. To disrupt this protein framework, researchers have focused their attention to proteolytic enzymes, namely, proteases, as potent antibiofilm agents, which catalyze the hydrolysis of peptide bonds in the protein components within the EPS.

Enzymes such as proteinase K (a broad-spectrum serine protease), trypsin, and subtilisin catalytically cleave peptide bonds, thereby breaking down protein structures. This degradation directly affects the integrity of the biofilm, dissolving the adhesive and structural proteins that attach the community to a surface and to itself. For instance, Karygianni et al.[39] revealed that treatment of oral biofilms with proteinase K induced a decrease in the activity of *C. albicans*, while biofilm thickness decreased from 28.5 μm (control) to 9.07 μm (0.05 mg/mL) and 7.4 μm (0.1 mg/mL). Similarly, Radhakrishnan et al.[40] demonstrated the removal of *S. aureus* biofilm after 12 h by serine protease isolated from *Solanum trilobatum*. Beyond degrading structural components, proteases also disrupt quorum sensing (QS) pathways by targeting signaling molecules and the proteins involved in their production and detection. Many bacteria depend on QS to coordinate biofilm development and the expression of virulence factors. Recently, Nahar et al.[41] have investigated the antibiofilm efficacy of Flavourzyme, a blend of exo- and endopeptidases, and revealed that 0.125 × minimum inhibitory concentration (MIC) of Flavourzyme inhibited > 4.5 log CFU/peg of biofilm. Cell motilities and the biosynthesis of QS molecules such as N-acyl-homoserine lactones (AHLs), including C4-HSL, decreased significantly at 0.06 × MIC of Flavourzyme and were undetectable at 0.125 × MIC. Interestingly, although 0.03 × MIC of Flavourzyme elicited diverse expressions of QS and virulence-regulating genes, ≥ 0.06 × MIC of Flavourzyme remarkably suppressed the relative genomic expressions. By interfering with these communication systems, proteases can prevent the transition from planktonic to biofilm growth modes and trigger the dispersal of established biofilms. This mechanism is particularly beneficial as it addresses both the formation and maintenance of biofilms without exerting selective pressure for traditional antibiotic resistance. In addition, neutrase (a bacterial protease from *Bacillus subtilis*) and alcalase (an alkaline protease) also demonstrated substantial antibiofilm activity. In a study conducted by Li et al.[42], neutrase achieved approximately 90.9% removal of *Shewanella marisflavi* biofilms at an initial concentration of 3×10^6 cells/cm^2, while alcalase treatment resulted in up to 50.14% bacterial death rates. The concentration-dependent efficacy of these enzymes was evident, with optimal activity observed at specific concentrations (8 g/L for neutrase and 6 g/L for alcalase). Overall, proteases exhibit a dual mechanism of action against foodborne biofilms by directly degrading the structural protein components of the matrix and by indirectly disrupting QS signaling pathways, which modulates bacterial communication and inhibits coordinated biofilm development. This combined action on both physical integrity and the regulatory systems of biofilms makes the use of protease enzymes a significant strategic tool for controlling persistent contamination in food processing environments.

Proteases offer distinct advantages over traditional chemical biocides. First, their mode of action is extracellular and enzymatic, indicating that they need not penetrate bacterial cells and therefore do not promote the development of genetic antibiotic resistance in the same way as traditional antimicrobials[43]. Second, they are generally regarded as safe, biodegradable, and environmentally friendly, aligning with the consumer and industry trends toward "green" cleaning technologies[4]. However, their deployment still

faces challenges. Biofilm composition is highly variable between bacterial species and is influenced by environmental conditions such as nutrient availability. A biofilm formed in a nutrient-rich medium may have a different protein profile from that formed under nutrient starvation, potentially affecting protease efficacy.

In processing plants, proteases have been specifically investigated for use on various surfaces, where bacterial attachment and biofilm formation pose direct contamination risks. For example, Fucinos et al.[44] validated the effectiveness of the enzymatic cleaning formulation using proteases in a real food processing industry. All the samples were analyzed with the sandwich R5 enzyme-linked immunosorbent assay (ELISA), and the results showed that gluten content always decreased under the detection limit (< 0.125 μg per 100 cm^2), in different equipment, namely, conveyor belt, dusting machine, and breading machine.

The tendency of high-concentration protease solutions to corrode certain metal surfaces, particularly sensitive alloys such as aluminum or carbon steel, represents a tangible yet manageable risk that must be carefully addressed during formulation and process design. Unlike strong acids or oxidizers, proteases are not inherently corrosive chemicals; however, their enzymatic activity requires specific operational conditions that can inadvertently create a corrosive environment. Fridrich et al.[45] stated that metalloproteinases require specific operational conditions, including neutral pH and metal cofactors like zinc, which can create a corrosive environment if not handled properly. Therefore, in addition to the enzyme itself being the primary corrosive agent, the combined chemical and physical conditions of its application also contribute, which must be assessed through material compatibility testing with specific equipment alloys.

Enzymes targeting biofilm lipids

Lipid-rich biofilms serve as reservoirs for major foodborne pathogens, continuously posing contamination risks that undermine food safety protocols. In meat processing plants, *Salmonella* can be contaminated during slaughter and processing that persist in biofilms on equipment, increasing the likelihood of cross-contamination of meat[46]. The hydrophobic lipid matrix physically protects these pathogens from aqueous sanitizers, thereby allowing them to escape cleaning cycles and subsequently recontaminate the upcoming fresh product. This persistence is directly linked to recurrent foodborne illness outbreaks and product recalls, with enormous public health consequences.

The composition of lipids in biofilms varies considerably between microbial species and is influenced by environmental conditions. Common lipid constituents, including phospholipids from cell membranes, fatty acids, glycolipids, and other hydrophobic compounds, contribute to the matrix's physical properties. These lipids are particularly abundant in the outer layers of biofilms, where they create a hydrophobic zone that limits penetration of aqueous solutions and contributes to the surface adhesion properties[47]. This strategic positioning makes lipids an attractive target for enzymatic degradation approaches aimed at disrupting biofilm integrity and increasing susceptibility to antimicrobial agents and host defenses. Recently, El-Naga et al.[48] have shown that *Bacillus subtilis* (DSM 1088)-derived lipase exhibits antibacterial effects against *S. aureus* with a MIC and minimal bactericidal concentration (MBC) of 1/8 and 1/16, respectively, confirming a bactericidal effect (MIC/MBC ratio ≤ 2). Biofilm inhibition assays demonstrated 95% biofilm reduction at 80 μg/mL lipase, with scanning electron microscopy (SEM) imaging revealing significant biofilm matrix disruption. In addition, Esakkiraj et al.[49] evaluated the antibiofilm efficacy of *Psychrobacter celer* PU3 lipase (PCL) against pathogenic *Vibrio parahaemolyticus* isolated from diseased shrimp, and found an MIC value of 500 U. PCL significantly altered the morphology and biofilm density of *V. parahaemolyticus*; this was observed through SEM and confocal laser scanning microscopy (CLSM) imaging. Reverse transcription polymerase chain reaction (RT-PCR) analysis revealed that the mRNA expression level of biofilm, colony morphology, and major toxin-related (*aphA, luxS, opaR, tolC, toxR*) genes of *V. parahaemolyticus* were significantly downregulated with PCL treatment.

Lipases (glycerol ester hydrolases, EC 3.1.1.3) represent the primary class of enzymes that can target and degrade lipid components within biofilm matrices. These enzymes catalyze the hydrolysis reaction of triglycerides into free fatty acids and glycerol, but many lipases also demonstrate activity against a broader range of substrates, including phospholipids, glycolipids, and other ester-containing compounds found in biofilms. The mechanism of action involves the attack of ester bonds in lipid molecules, resulting in the breakdown of the lipid components that contribute to the integrity and hydrophobicity of the biofilm matrix[47]. This degradation process disrupts the protective barrier function of the lipid-rich outer layers, thereby increasing the biofilm's permeability to antimicrobial agents and facilitating deeper penetration of other EPS-degrading enzymes.

The action of lipases against biofilms is influenced by several factors, including enzyme concentration, pH, temperature, and the specific lipid composition of the target biofilm. The optimal activity for most bacterial lipases occurs at neutral to alkaline pH values (pH 7.0–9.0) and temperatures between 30 and 40 °C; however, these parameters vary depending on the enzyme source[46]. The structural complexity of biofilm matrices may also affect lipase efficacy, as the enzyme must penetrate the outer layers to access lipid components distributed throughout the EPS. This challenge in penetration may explain why lipases often demonstrate greater efficacy when combined with other EPS-degrading enzymes that can facilitate matrix penetration.

Enzyme cocktails

The structural resilience of biofilms largely depends on the complex, heterogeneous nature of the EPS, which is a composite matrix of polysaccharides, eDNA, proteins, and lipids[7,8,9]. This heterogeneity presents a significant challenge for monotherapeutic approaches. An enzyme targeting a single polymer, such as DNase attacking eDNA or a protease degrading proteins, often leads to incomplete dispersal. The remaining intact EPS components can maintain sufficient structural integrity to continue protecting embedded cells, allowing rapid regeneration of the biofilm. This limitation is particularly evident in mixed-species biofilms, which are the norm in environmental settings such as food processing plants, where different microbes contribute varying EPS components[50]. These biofilms, formed by synergistic microbial interactions, demonstrate enhanced resistance to disinfectants and antimicrobials compared to single-species biofilms or planktonic cells.

To overcome this, a leading trend in biofilm remediation is the development of specifically designed enzyme cocktails to attack the matrix on multiple fronts simultaneously (Fig. 2). Research indicates that combinations of enzymes such as DNase I with a broad-spectrum protease like proteinase K, or alongside glycoside hydrolases

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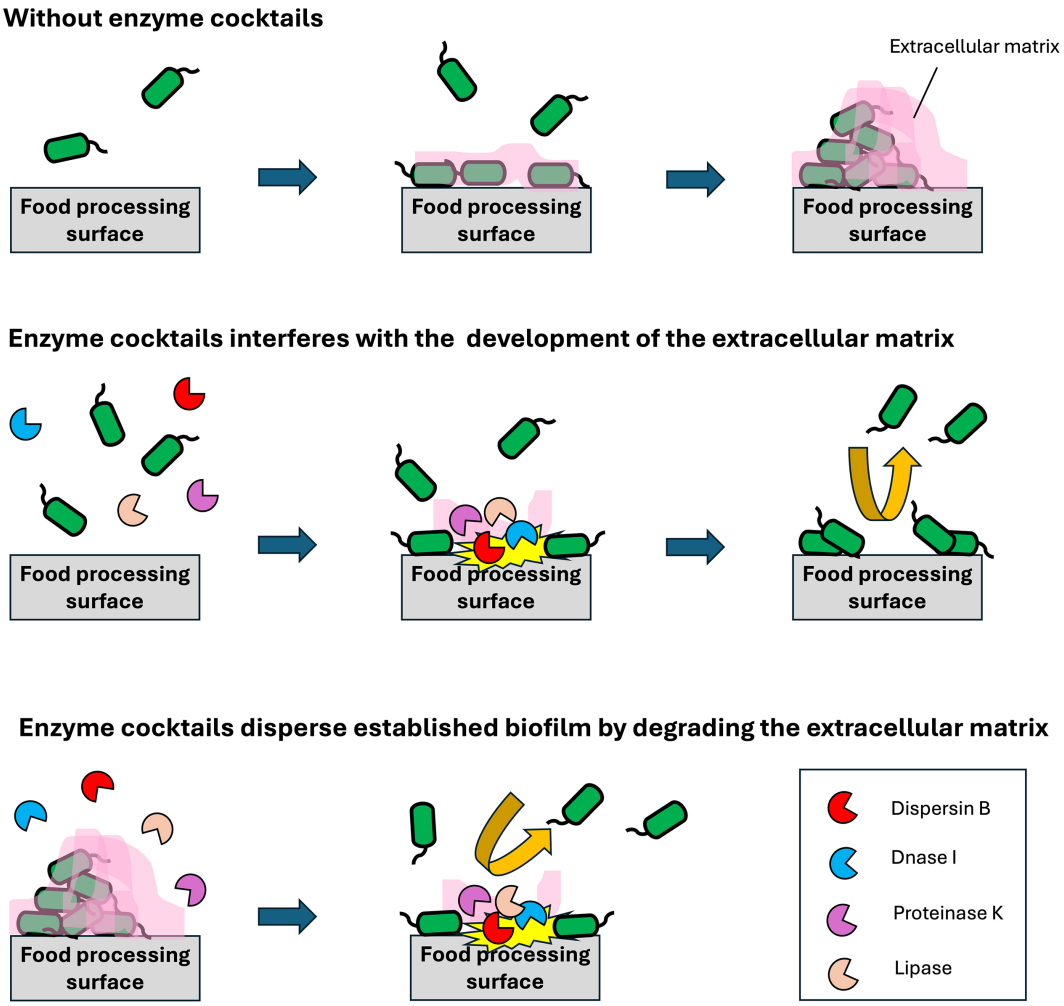

Fig. 2 Synergy between enzymes targeting the extracellular matrix of foodborne biofilms.

that target specific polysaccharides like cellulose or β-glucans, act synergistically. By degrading eDNA, protein adhesives, and sugary polymers in parallel, these cocktails lead to a comprehensive failure of the architectural integrity of the biofilm. Studies on multispecies consortia have demonstrated that these multienzyme formulations are more effective than single enzymes, especially against mature biofilms that develop a highly cross-linked and stratified EPS structure. For example, Nielsen et al.[51] evaluated > 400 different treatment modalities, using 44 different enzymes in combinations with up to six enzymes in each formulation, on *in vitro* biofilms inoculated with human saliva. They found that multienzyme formulations removed up to 69% of the biofilm volume, and the most effective formulations all contained mutanase, an enzyme that breaks down α-1,3-glucans, a major cell wall polysaccharide in filamentous fungi.

The strategic value of enzymes extends beyond standalone treatment, serving as a powerful primer for conventional chemical disinfectants. The primary barrier to the efficacy of a disinfectant is its poor penetration through the dense, protective EPS[52]. Enzymatic pretreatment functions as a "matrix disruption phase" in a cleaning protocol[53]; by disrupting the EPS scaffold, enzymes create channels and breaches that allow disinfectants, such as chlorine, quaternary ammonium compounds, or peracetic acid, to infiltrate deeply and make direct contact with the now-exposed microbial cells. This sequence produces a powerful synergistic effect, where the physical removal action of the enzymes is combined with the lethal biocidal action of the disinfectant. This combination strategy offers a pragmatic pathway for the food industry to address several significant operational and environmental concerns. Meanwhile, machine learning (ML) enables systematic optimization of enzyme blends by predicting optimal enzyme combinations and their synergistic properties. For example, Akgüller et al.[54] developed an ensemble ML framework that integrates kinetic parameters, sequence features, and network topology to identify optimal enzyme combinations for plastic degradation. Their multiobjective optimization algorithm evaluated enzyme pairs across four criteria (prediction confidence, substrate coverage, operational compatibility, and functional diversity), identifying 156 Pareto-optimal enzyme combinations with composite scores exceeding 0.89. Studies on the antibiofilm efficacy of single enzymes and multienzymes are summarized in Table 2.

Delivery systems for enzymatic dispersal

Proteases, DNases, and glycoside hydrolases are inherently fragile biomolecules. Their tertiary structures, essential for catalytic activity, are prone to denaturation from the high temperatures used in clean-in-place (CIP) systems, extreme pH levels from alkaline or acidic cleaning agents, and inactivation by surfactants or oxidizing

Table 2. Single enzymes and multienzymes targeting the biofilm matrix.

Enzymes	Antibiofilm efficacy	Ref.
Protease, polysaccharidase	Proteolytic enzymes promote biofilm removal in a diverse range of bacterial species compared to polysaccharidases. Serine proteases are more efficient in removing cells of *Bacillus* biofilms than polysaccharidases. However, polysaccharidases are more efficient in removing *P. fluorescens* biofilms than serine proteases.	Lequette et al.[55]
DNase I, pronase, pectinase	CLSM images show significant changes in *Listeria monocytogenes* biofilm-covered area and volume after DNase I, pronase, and pectinase treatments.	Puga et al.[56]
Protease, amylase	Pancreatic protease exhibits significant antibiofilm effects against *S. aureus*, Methicillin-resistant Staphylococcus aureus (MRSA), and *E. coli*. Pancreatic protease combined with bacterial amylase exhibits enhancement of the antibiofilm effects against *S. aureus* and MRSA biofilms.	Jee et al.[57]
Protease, DNase I	Treatment with Flavourzyme shows a significant reduction of young (24-h-old) and mature (72-h-old) biofilms on both ultra-high-molecular-weight polyethylene (UHMWPE) and rubber surfaces. The overall reduction potential of Flavourzyme was higher than that of DNase I. The Flavourzyme-mediated removal of biofilms appears to be caused by the gradual disruption of amide and polysaccharide stretching bands of the extracellular polymeric substances (EPSs) released by the microbes.	Nahar et al.[58]
Hydrolase	0.1 mg/ml of glycosyl hydrolases inhibits up to 41% of biofilm formation by *Escherichia coli* O157:H7, *E. coli* 25922, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes*. Enzyme treatment of all four cell types results in significantly reduced cell surface hydrophobicity and collapse of *E. coli* 25922 cells imaged by electron microscopy.	Mayton et al.[59]
Lipase, cellulase, protease	The combined enzymes (lipase, cellulase, and proteinase K) significantly inhibit the development of *Vibrio parahaemolyticus* biofilm. The confocal laser scanning microscopic images confirm that the aggregation of microcolonies and the adhesion of biofilm are inhibited with the combined enzyme treatment. Furthermore, combined enzymes also decrease the concentration of exopolysaccharide (EPS) and disrupt the EPS matrix network.	Li et al.[60]
Dispersin B	Dispersin B increases the susceptibility of *S. epidermidis* biofilm cells to ciprofloxacin	Abdelkader et al.[61]
Protease, amylase	SL40, a commercial detergent product containing subtilisin protease and α-amylase glycosidase, removes up to 85% of the biofilm biomass compared to tris solutions. SL40's efficacy was strongly influenced by the presence of the enzymes and both temperature and concentration.	Le Sénéchal et al.[62]
Polysaccharidase	Treatment with a mixture of engineered polysaccharide-degrading enzyme (CAase) and bacteriocin (thermophilin 110) yields similar results to treatment with CAase alone, demonstrating that the combination of thermophilin 110 and CAase does not enhance or inhibit the removal of the biofilm relative to CAase alone.	Renye et al.[63]

chemicals present in commercial detergents[64]. This inherent instability leads to a rapid loss of efficacy, making direct application of free enzymes in these settings both economically impractical and functionally unreliable. To overcome these formidable barriers, a major focus of applied research is on optimizing advanced delivery systems designed to protect enzymes from these denaturing conditions.

The mechanisms of enzyme delivery to disrupt biofilm matrix are shown in Fig. 3. The simplest mechanism involves formulating enzymes in a buffer or solution for direct application to the biofilm, such as irrigation for wound care or flushing for industrial systems[65]. The enzymes then diffuse into the biofilm matrix, thereby degrading its structural components. The major drawbacks of this approach are the poor stability of free enzymes in complex environments and their likely rapid removal from the site of application, which can reduce efficacy. A highly promising approach is the encapsulation of enzymes within protective matrices, such as biodegradable polymers, silica nanoparticles, or liposomes. These micro- or nanoscale capsules act as protective shells, physically insulating the enzyme from detrimental factors such as proteolytic degradation or pH shocks while still allowing the substrate (e.g., eDNA or proteins) to diffuse in and be broken down. For example, Zhang et al.[66] demonstrated the high efficiency of enzyme encapsulation in silica nanocapsules using an amphiphilic precursor polymer, poly(ethylene glycol) substituted hyperbranched polyethoxysiloxane (PEG-PEOS), in aqueous conditions, achieving ~50% encapsulation efficiency with only 5 wt% polymer while preserving 40% of protease activity and enabling repeated regeneration. Similarly to encapsulation, the immobilization of enzymes onto solid substrates provides a complementary strategy for creating stable, reusable antibiofilm surfaces. Instead of being added to a cleaning solution, enzymes are covalently bound or adsorbed onto materials like modified cellulose, stainless steel, or polymer nanofiber mats. This technique provides multiple advantages: the immobilized enzyme is typically more robust to temperature fluctuations and can be rinsed and reused multiple times, making it drastically cost-effective. Elchinger et al.[67] reported that proteases immobilized on chitosan films exhibit strong antibiofilm activity against *S. aureus, L. monocytogenes*, and *P. aeruginosa*, as well as high immobilization efficiency and effective inhibition of biofilm formation. Similarly, immobilized pectinase and subtilisin A decreased *E. coli* biofilm biomass and thickness at sublethal concentrations and synergistically enhanced antibiotic efficacy[68].

Application in food processing environments

The implementation of enzymatic biofilm dispersal strategies in the food industry is complicated by unique and interconnected practical constraints. The diversity of surface materials, including stainless steel, polyethylene, rubber seals, and glass, presents a fundamental challenge, as each surface exhibits distinct topographical and chemical properties that determine the adhere of biofilms and, consequently, the effectiveness of enzyme penetration and disruption[69]. Furthermore, any new cleaning regime must navigate a complex balance between antimicrobial efficacy, strict food safety regulations regarding residue, overall operational costs, and the practical ease of integration into existing workflows without any drawbacks. This requires formulations that are not only potent but also stable, noncorrosive, and safe for use in environments where product contamination is a constant concern. Despite these significant drawbacks, a growing body of proof-of-concept and pilot-scale studies provides compelling evidence for the feasibility of enzymatic treatments. In the dairy industry, for example, where the presence of heat-stable biofilms composed of proteins and fats is a major issue, cleaning solutions added with proteases and amylases have been successfully tested[70]. These enzymes specifically target

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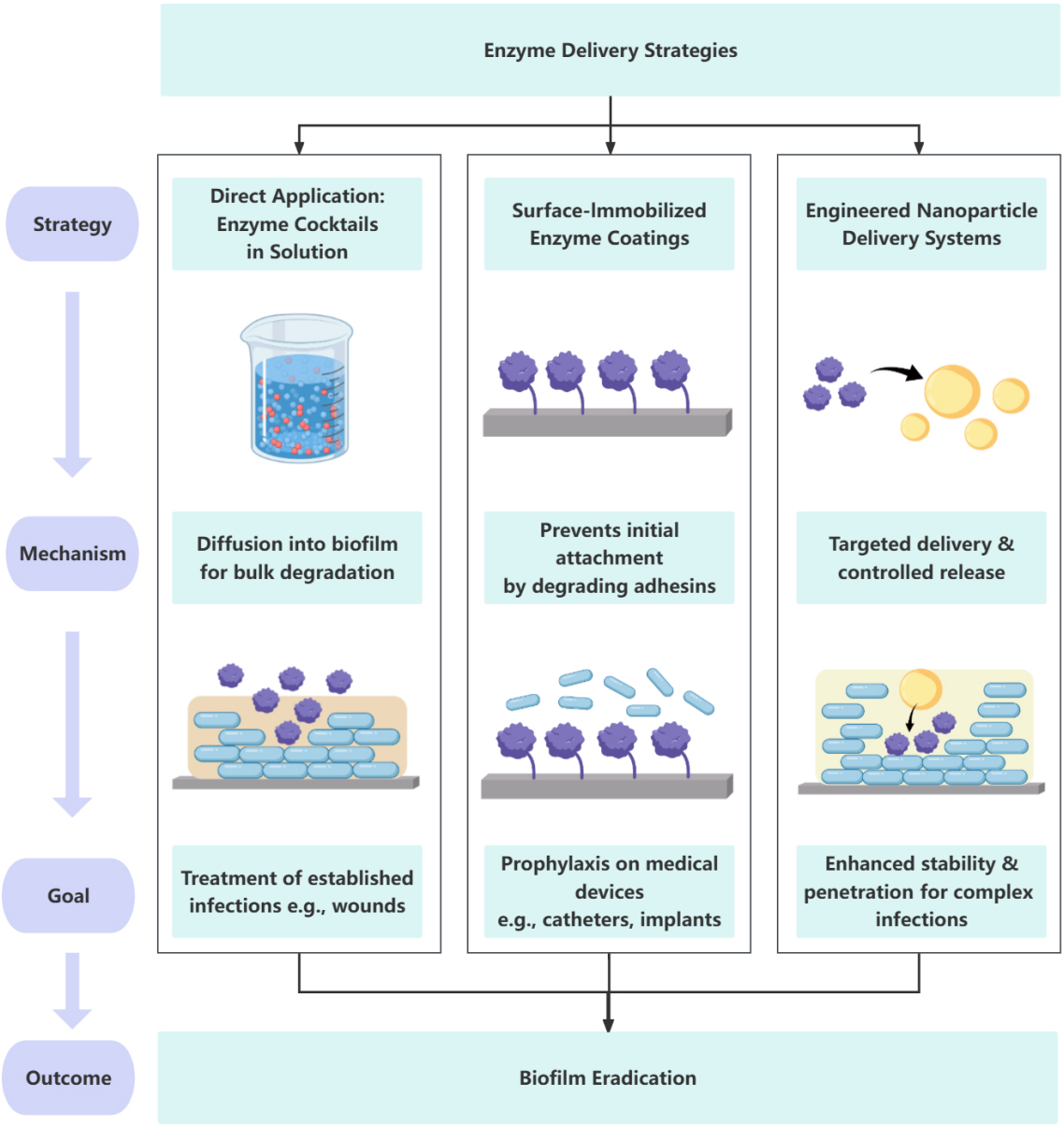

Fig. 3 Mechanisms of enzyme delivery to disrupt the biofilm matrix.

the organic load, breaking down the proteinaceous and sugary matrix that shields microbial cells.

Interestingly, enzymes in the food industry serve the dual purposes of enhancing both food safety and food quality (Fig. 4). On the one hand, as discussed, enzymes function as potent antibiofilm agents, helping to control microbial contamination and thereby improving food safety. On the other hand, many of the same classes of enzymes, particularly proteases and lipases, are extensively used to improve the palatability, texture, and overall sensory attributes of food products[71]. Moreover, plant-derived cysteine proteases enhance the texture and palatability of meat while offering nutritional benefits and enabling value addition to low-grade meat cuts[72]. Other studies have also shown that proteolytic enzymes are well-established components in food processing, with a long history of safe use in applications such as meat tenderization (papain, bromelain), cheese production, and baking[73,74]. This dual application is a proof to the versatility of enzymes in the food industry.

Safety, regulatory, and economic considerations

The transition of enzymatic biofilm control from a promising laboratory concept to a mainstream industrial tool depends on the successful navigation of a triad of critical considerations: human and product safety, regulatory approval, and economic viability. Although many enzymes are classified as Generally Recognized As Safe (GRAS) for their intended uses in food processing, their application as cleaning and dispersal agents introduces new risk profiles that must be thoroughly evaluated[75]. This necessitates a comprehensive assessment of their potential toxicity and allergenicity upon incidental ingestion, inhalation by workers, or skin contact during handling. Regulatory frameworks in major jurisdictions like the European Union (governed by EFSA) and the United States (overseen by the FDA) have drawn a clear distinction between enzymes used as direct food additives and those used in processing aids or cleaning agents[76]. Enzymes intended for incorporation into food-contact

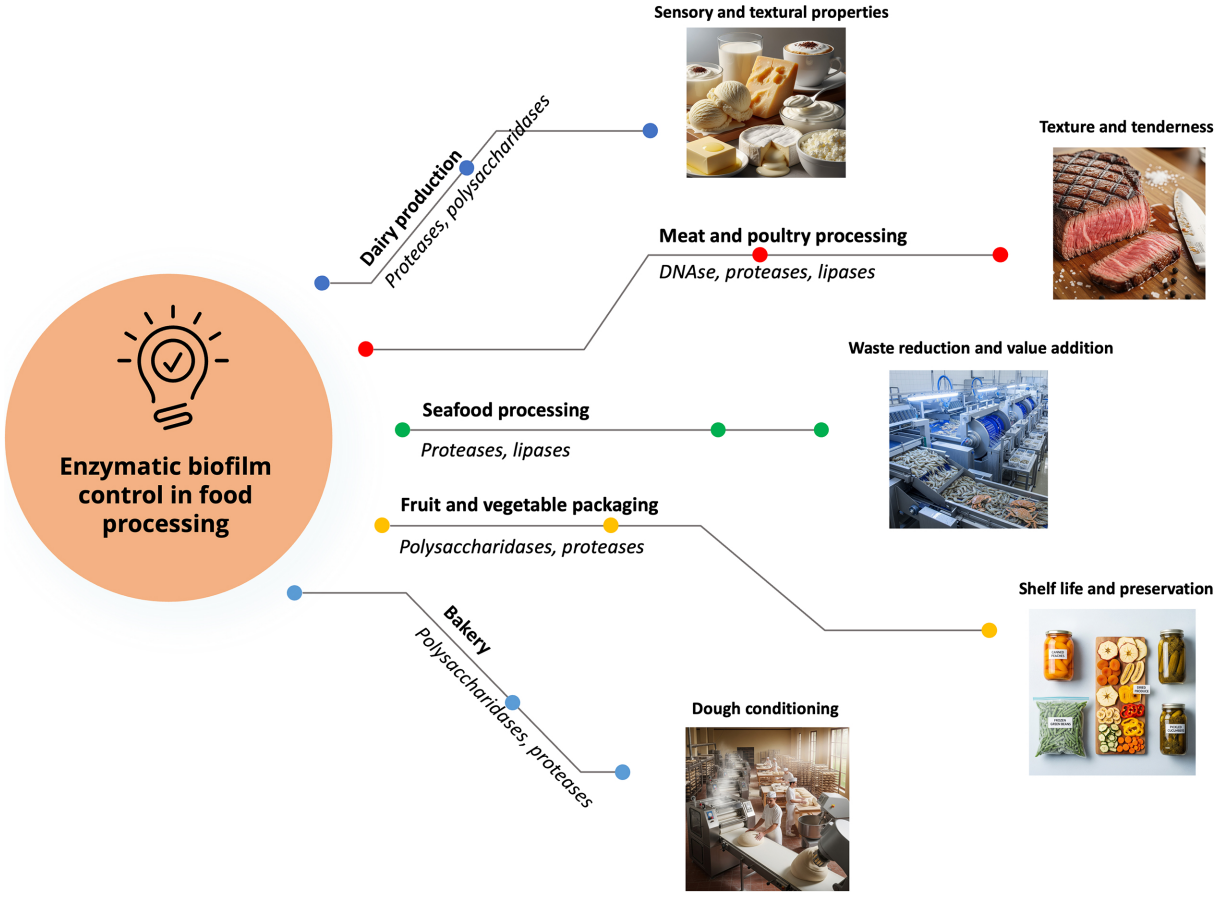

Fig. 4 The dual role of enzymes: safeguarding safety and enhancing quality in the food industry.

materials or packaging undergo a rigorous pre-market safety assessment and approval process. However, for enzymes used in cleaning-in-place (CIP) systems that are thoroughly rinsed off prior to production, the regulatory pathway is less strict if the manufacturer demonstrates that no functionally significant residues remain on the surface.

A primary drawback that remains, even after establishing safety and regulatory compliance, is achieving cost-effectiveness. The production, purification, and stabilization of high-quality food-grade enzymes incur a significant cost, often making enzymatic formulations a more expensive upfront than traditional chemical disinfectants like chlorine or quaternary ammonium compounds[77]. This cost constraint is a major hurdle for an industry operating on thin margins. However, this economic calculus has been changing due to several technological advances. The use of recombinant expression systems in microbial hosts allows for the high-yield and consistent production of specific enzymes[78]. More importantly, strategies like enzyme immobilization on reusable solid supports or encapsulation for controlled release can significantly reduce the amount of enzyme needed per cleaning cycle by protecting it from denaturation, thereby allowing its repeated use[79,80]. When the need for reduced chemical usage, longer equipment lifespan due to less corrosion, and improved sustainability are considered in the total cost of ownership, the value proposition of enzymatic strategies becomes increasingly compelling, paving the way for their broader adoption in food safety protocols.

Cleaning operations are a major factor for water consumption in food processing plants, with conventional sanitation often requiring multiple rinse cycles to remove chemical residues between alkaline and acid steps. Enzymatic cleaning significantly reduces this water consumption through several mechanisms. First, enzymes function effectively in reduced water volumes because their catalytic activity does not depend on dissolving large quantities of chemical detergents; they remain active at lower concentrations and can penetrate biofilms more efficiently in environments with minimal liquid[81]. Second, the compatibility of many enzymatic formulations with subsequent sanitizer application[51] reduces or eliminates the need for intermediate rinsing, allowing integrated cleaning steps that consolidate water usage. Third, because enzymes degrade biofilm matrix components[5] rather than simply solubilizing surface soils, less mechanical rinsing is required for the physical removal of debris; the disrupted biofilm fragments are more easily suspended and carried away in shorter, lower-volume rinse cycles.

Conventional cleaning in the food industry is inherently energy-intensive, depending on elevated temperatures to activate chemical reactions, melt fats, and enhance soil removal. Hot water generation for CIP systems, steam for equipment sanitation, and heated rinse solutions collectively represent a major fraction of a processing plant's thermal energy budget. Enzymatic cleaning fundamentally changes this energy equation because enzymes function optimally at moderate temperatures[15], typically between 30 and 50 °C, matching the ambient conditions of many food processing environments. This eliminates the need for energy-intensive heating of cleaning solutions, allowing processing plants to achieve superior biofilm removal using ambient or modestly warmed water. Furthermore, the reduced contact times enabled by enzymatic catalysis mean that pumps and circulation systems operate for

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shorter durations, which decreases the consumption of electrical energy.

The carbon footprint of industrial cleaning operations encompasses emissions from multiple sources: fuel combustion for water heating, electricity for pumping and circulation, chemical manufacturing and transportation, and wastewater treatment. Enzymatic cleaning attenuates emissions across this entire lifecycle. Reduced thermal energy demand directly lowers combustion emissions from boilers and water heaters. Decreased pumping requirements reduce grid electricity consumption and its associated emissions. Furthermore, enzymes are typically shipped and stored as concentrated liquids or stable powders, concentrating their functional activity into smaller transport volumes with lower associated logistics emissions. With the above factors together with lifecycle assessment methodologies, enzymatic cleaning protocols can demonstrate significant reduction in carbon footprint. According to Molina-Espeja et al.[82], enzymes can lead to an annual decrease of CO_2 emissions by 1–2.5 billion tons, carbon demand by about 200 million tons, and chemical demand by about 90 million tons.

Conclusions

Biofilms pose a persistent challenge in the food industry, where their resistance to cleaning and disinfection undermines food safety and increases costs. Enzymatic dispersal offers a targeted, biodegradable, and potentially cost-effective strategy to complement or replace traditional sanitation methods. Enzymes such as glycoside hydrolases, DNases, and proteases have demonstrated strong antibiofilm activity, particularly when used in synergistic combinations or as pretreatments to conventional disinfectants. Advances in enzyme delivery systems, including encapsulation and immobilization, improve stability and expand practical applications. However, regulatory approval, economic feasibility, and standardization remain critical barriers to the commercialization of enzymes. In the near future, enzymatic dispersal is likely to play an increasing role in sustainable biofilm management, especially as industries aim at reducing chemical usage and improving hygiene. Continued interdisciplinary research bridging microbiology, food engineering, and materials science is essential for the successful transmission of laboratory findings into robust industrial solutions. With the growing awareness of biofilm-associated risks, enzymes represent a promising frontier in the quest for safer, cleaner, and more sustainable food production systems.

Author contributions

The authors confirm their contributions to the paper as follows: conceptualization: Yahya MFZR, Azmi MA, Zhang Q; original draft preparation: Yahya MFZR, Azmi MA, Zhang Q; review and editing: Jalil MTM, Zhang Q, Yahya MFZR, Azmi MA. All authors have read and agreed to the published version of the manuscript.

Data availablity

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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