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Novel chemical-based approaches for biofilm cleaning and disinfection

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Biofilm formation plays a critical concern in the food processing industry due to potential environmental, economic, and food spoilage impacts. To maintain microbial safe levels, a sanitation process, comprising cleaning and disinfection steps, needs to be periodically performed. Biocide treatment for surface disinfection is the most widely applied strategy. Owing to the critical failure of current sanitation processes, the microbial persistence on surfaces, and the events of resistance and cross-resistance, the search for new effective antimicrobial strategies is emerging. This review aims to provide an overview of emerging strategies for biofilm prevention/control, as alternatives to current cleaning and disinfecting agents. Specifically, the use of antimicrobial peptides, biosurfactants, bacteriophages, essential oils, extracellular polymeric substances, matrix-degrading enzymes, and nanoparticles as alternative strategies for biofilm control in the food industry was revised, focusing on their main applications, advantages, and limitations. Combinatory approaches were critically assessed for allowing a personalized and specific attack of microbial contaminations, which may have a significant relevance in improving biofilm control while reducing the environmental impact from the use of conventional biocides.

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Introduction

Microorganisms have the ability to form biofilms, which are characterized by a highly dense microbial community (surface-attached, interface-attached, or attached to each other) embedded in a self-produced matrix of extracellular polymeric substances (EPS) [1]. In industrial settings, biofilms are responsible for several problems, including economic loss, (bio)corrosion, reduction of process and product microbial safety, and potential public health impacts. Additional operating and maintenance costs are a consequence of high-energy consumption (reduction of heat and mass transfer efficiency), the cost of additives (such as antimicrobial agents), and unplanned shutdowns for sanitation (cleaning and disinfection). In the food processing industry, biofilms are of particular concern due to the potential presence of pathogenic and spoilage microorganisms [2].

The biofilm state is associated with increased protection from external stresses (i.e. hydrodynamics, desiccation, antimicrobial agents, and protozoan grazing) [1]. This increased antimicrobial tolerance is attributed to multiple intrinsic factors: diffusion-reaction limitations through interaction with EPS components, low cell accessibility, production and accumulation of degradative enzymes and neutralizing chemicals, microenvironments and phenotypic heterogeneity (nutrient and oxygen depletion), cell-to-cell communication — quorum-sensing (QS) system, low metabolic activity with reduced biocide uptake, presence of persister cells, and enhanced horizontal gene transfer [3]. Given the systematic failure of current sanitation procedures and the resilience of biofilm-colonizing microorganisms, the development of new effective strategies for industrial surface disinfection is in demand. Since biofilms confer strong protection against sanitation, the best strategy should be their prevention through regular disinfection, which is an unreachable target [4]. In addition to the biofilm tolerance being well-recognized, there is a conspicuous absence of novel and effective control strategies [5]. The use of new biocides has become more critical especially in Europe, as a result of the Biocidal Product Directive and Directive 98/8/EC of the European Parliament and the Council, which have decreased the available biocides for industrial application and declined the launch of new active molecules [6]. A strategy of potential interest to replenish the biocide pipeline is the off-label use of chemicals currently applied in industrial products and

processes, and their combination with already-approved biocides to improve the antimicrobial activity of the active molecules [5]. This review provides insights into emerging disinfection strategies for biofilm prevention and control. Aspects of antibiofilm effectiveness, development of antimicrobial resistance, and the environmental impact of sanitation are also considered.

Antibiofilm strategies

Biofilm prevention or control of pre-established biofilms (i.e. killing and removal from surfaces) is currently attained by sanitation. The selection of sanitation practices depends on the process conditions, such as type of surface material, biocide (type and concentration), exposure time, and microbial contaminants. It is advised to apply recommended concentrations, temperatures, and exposure times set by the manufacturers of the cleaning and disinfection products [4]. Additionally, high antimicrobial effectiveness is ensured by repeated cleaning cycles with a serial application of more than one type of antimicrobial agent and high antimicrobial dose and/or exposure time (critical biocide treatments) [4]. Nevertheless, high shutdown time, antimicrobial cost-effectiveness, human adverse reactions from chemical exposure (staff risk), and equipment corrosion must be considered before implementing such procedures [4].

Biocide treatment is widely used for surface disinfection, aiming to prevent and control biofilms. A wide range of in-use biocides is available, including halogen-releasing, peroxygen, organic acid, aldehyde, quaternary ammonium compounds (QACs), and alcohol-based compounds [7]. Research efforts have been made to understand the mode of action of biocides. Their antimicrobial activity is typically related to the chemical nature and concentration of biocides, environmental conditions (organic load, pH, and temperature), bacterial diversity, and disinfection protocol (pre cleaning and exposure time) [8,9]. Table 1 summarizes the main classes of biocides and their mode of action, as well as the main advantages and drawbacks of their use. It is important to highlight the factors affecting biofilm properties (i.e. cell density, structure, mechanical properties, growth rate, and expression of virulence factors), which also impact sanitation (Figure 1). For example, Simões et al. [10] reported that high shear stress stimulated the formation of cell-denser and EPS-poor biofilms, where a basal viable layer remained after biocide treatment in comparison to biofilms formed under low shear stress. Furthermore, *Bacillus cereus* and *Pseudomonas fluorescens* biofilms formed on high-density polyethene (HDPE) and stainless steel (SS) were affected at comparable extents to cleaning and disinfection, while those formed on polymethylmethacrylate (PMMA) were more tolerant to a QAC and mechanical treatments [11]. The antibiofilm effects were also species-specific, where peracetic acid (PAA) was more efficient against *P. fluorescens* (2.3-log reduction), followed by sodium hypochlorite and chlorhexidine gluconate (0.7–1.0-log reduction), while no difference was obtained against *Pseudomonas aeruginosa* (1.1–1.8-log reduction) [2]. The antibiofilm effectiveness is mainly assessed in terms of biofilm inactivation, inhibition, or removal. However, Ledwoch et al. [12] demonstrated that the reduction of cell viability was not enough to provide information about antibiofilm effects, since sanitation did not prevent biofilm recovery, and detached bacteria can be transferred to other surfaces, reseeding a new biofilm. Biofilm regrowth events were rarely reported and can impose high selective pressure for the development of antimicrobial tolerance. Castro et al. [2] demonstrated that disinfection following the manufacturer's instructions did not cause complete *Pseudomonas* sp. biofilm eradication from SS surfaces (under conditions mimicking the dairy processing plant), and the recovered biofilms showed increased tolerance. Attending all these factors affecting sanitation efficacy, it is of utmost importance to identify and characterize the main contaminants of the process to select a sanitation strategy adjusted to the process needs. Several mathematical models have been used to simulate and predict biofilm formation and its behavior under different conditions (i.e. adhesion surface roughness, physical and chemical properties, temperature, pH, bacterial diversity, salinity, and nutrient availability) [13]. These models may constitute an important strategy to swiftly obtain information about biofilms in a specific equipment/process and adjust an optimized disinfection strategy for biofilm eradication. Predictive models have been applied for safer food storage [13]. However, the high variability on operational conditions among processes and products difficult the precise use of these predictive mathematical models.

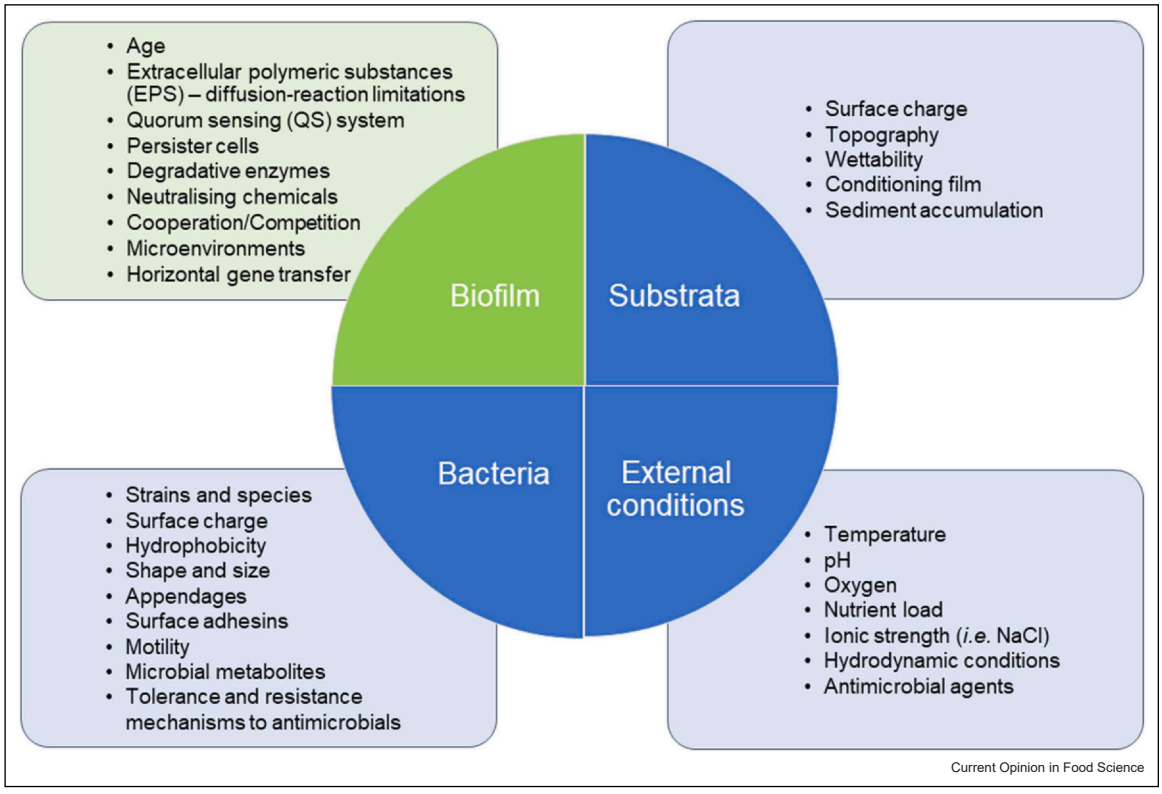
Besides biocides being used for surface disinfection and biofilm eradication, physical methods have also been applied for this purpose or even to improve biofilm control in combination with biocides. Hot steam application, ultrasonication, ultraviolet (UV) irradiation, high hydrostatic pressure, and surface modification are examples of physical methods that can be applied in the food industry for biofilm control, and their efficacy may be improved when combined with chemical strategies [14]. Ultrasounds have been used in combination with chlorine-based disinfectants, organic acids (lactic acid and acetic acid), surfactants, or with electrolyzed water for improved microbial control in food-related surfaces or in fresh produce as reviewed by several authors [15,16]. Also, UV-C irradiation has been combined with oxidizing biocides for microbial control in fresh produce facilities [17]. The combined use of UV light (at different wavelengths) with multiple biocides (chloride, hydrogen peroxide, PAA, lactic acid, surfactants, and essential oils [EOs]) has been applied for microbial

Table 1

Mode of action, antibiofilm effects, and main advantages/disadvantages for each biocide group.

Group	Examples	Mode of action	Antibiofilm effects	Advantages/disadvantages	References
Halogen-releasing compounds	Calcium hypochlorite Chloramine-T Chlorine dioxide Sodium hypochlorite	Complete spectrum of action (bacteria, viruses, yeasts, fungi, and spores) Oxidative effect Disruption of cell integrity Interference with metabolic pathways	Loss of antimicrobial activity by chemical interaction with EPS Different adaptative responses in biofilm formation in a bacteria-dependent manner Chlorine dioxide gas — high diffusion across the biofilm	Cost-effective at low concentrations Corrosive Disinfection-by-product production Human hazardous Unstable in the presence of metals, UV radiation, heat, and organic load	[61–63]
Peroxygen-based compounds	Hydrogen peroxide PAA	Complete spectrum of action Oxidative effect Disruption of cell integrity Interference with metabolic pathways	Active in the presence of organic load Neutralization by catalase and peroxidases Diffusion across biofilm Cell inactivation without biofilm removal	Safe decomposition products Environmentally friendly Corrosive Pungent irritant smell Unstable in the presence of metals	[64–66]
Organic acid-based compounds	Acetic acid Citric acid L-lactic acid Phenyl-lactic acid Propionic acid	A broad spectrum of action Intracellular pH reduction Cellular osmotic stress	Disruption of EPS matrix	Low toxicity (GRAS status) Environmentally friendly High concentration required Low cost	[67–69]
Aldehyde-based compounds	Glutaraldehyde Glyoxal	A broad spectrum of action Reaction with amino acid residues of proteins mainly at alkaline pH	Loss of antimicrobial activity by chemical interaction with EPS Low diffusion across the biofilm Cross-linking agent — promoting microbial adhesion and mechanical biofilm stability	Noncorrosive Human hazardous	[70–73]
Quaternary ammonium components	BAC Cetrimonium chloride Cetylpyridium chloride Dodecyl-dimethyl-ammonium chloride Stearalkonium chloride	A broad spectrum of action Disruption of cell integrity (cell membrane destabilization and solubilization)	Active in the presence of organic load Low diffusion across the biofilm Bacterial inactivation without biofilm removal	Environmentally friendly Chemical stability Low corrosion Low biodegradability Resistance promotion	[74–77]
Alcohol-based compounds	Ethanol Isopropanol Ortho-phenylphenol 2-Phenoxyethanol	Denaturation of proteins Disruption of cell integrity Interference with metabolic pathways	Biofilm removal without its inactivation Adaptative response as high biofilm formation	High amounts required Resistance promotion Evaporation reduces contact time Highly flammable	[78,79]

Figure 1



The interplay between biofilm, substratum, bacterium, and external condition properties that affect biofilm formation and its control (surface disinfection).

control in fruit and vegetables as summarized by Mendoza et al. [16]. Also, Shahbaz et al. [18] demonstrated that UV–TiO_2 photocatalysis pre-washing followed by high hydrostatic pressure post package treatment can be an effective strategy, an alternative to conventional chlorine disinfection, for the inactivation of *Salmonella* on fresh cherry tomatoes. Moreover, it is important to highlight that conventional biocides used for food industry sanitation may have a significant environmental impact and may not be effective in biofilm eradication, due to the development of tolerance and resistance to these molecules. Therefore, there is a global need for new, more effective, and eco-friendly approaches, which may have a directed action on specific microorganisms colonizing the surfaces, contributing to the reduction of the selective pressure in the food industry. Also, the combination of different strategies should be further studied to develop advanced approaches with reduced environmental impact, from the use of low doses of biocides.

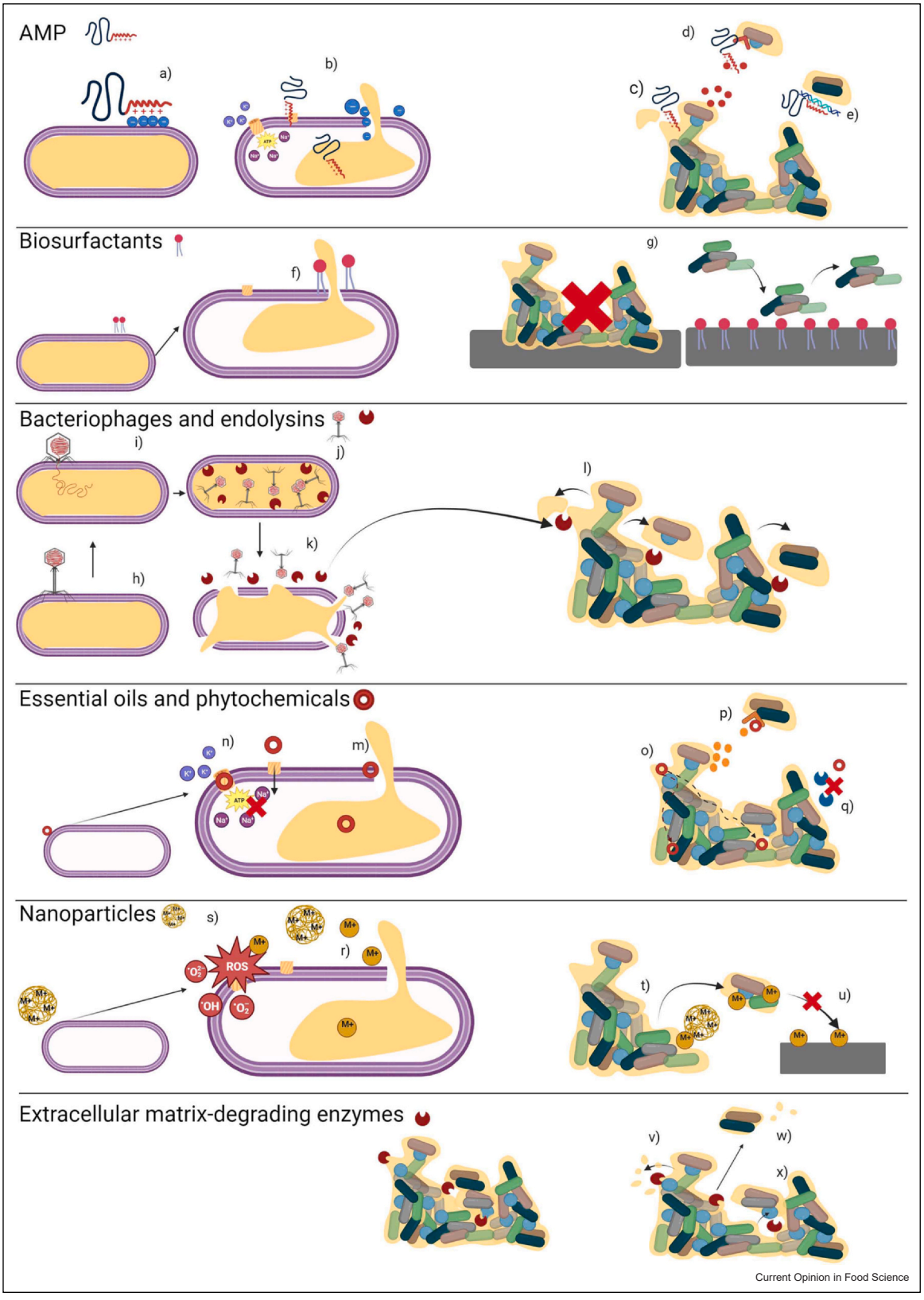
New antimicrobial strategies

Considering the limited antibiofilm effectiveness, production of toxic by-products, and the arise of biocide-tolerant strains, new antimicrobial alternatives have been extensively investigated to replace the use of conventional biocides [5,19]. Moreover, much pressure has been imposed by consumers and legal authorities linked to the industrial sector focused on adopting more natural alternatives for disinfection. Over the past decade, researchers have looked for sustainable and environmentally friendly, harmless to the One Health trilogy (humans, animals, and the environment), and effective alternatives to improve surface disinfection in the food processing industry [20]. Among the novel strategies used for disinfection, emerging efforts use natural agents, such as antimicrobial peptides (AMPs), biosurfactants, bacteriophages and endolysins, EOs and other phytochemicals, extracellular matrix-degrading enzymes, and nanoparticles (NPs). These strategies will be further described with a focus on their effectiveness in biofilm prevention/control. Figure 2 summarizes the mode of action on bacteria and biofilm control of all mentioned strategies. Table 2 summarizes their main applications in the food industry as well as their main advantages and limitations.

Antimicrobial peptides

AMPs are short cationic peptides (10–40 amino acid residues) involved in innate defense mechanisms against

Figure 2


AMP
a)
b)
c)
d)
e)
Biosurfactants
f)
g)
Bacteriophages and endolysins
i)
j)
k)
l)
h)
Essential oils and phytochemicals
n)
m)
o)
p)
q)
Nanoparticles
s)
r)
ROS
t)
u)
Extracellular matrix-degrading enzymes
v)
w)
x)
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Mechanisms of action of AMPs, biosurfactants, bacteriophages and endolysins, EOs and phytochemicals, NPs, and extracellular matrix-degrading enzymes in bacteria and biofilm control. **(a)** Interaction between positively charged AMP and the negative charge of bacterial surface, **(b)** resulting in the formation of pores in the membrane causing the leakage of intracellular components and disrupting the membrane potential. **(c)** AMPs degrade biofilm EPS, **(d)** interrupt the bacterial cell signaling, and **(e)** inhibit motility genes. **(f)** Biosurfactants disrupt cell membrane and form pores causing the leakage of intracellular components and **(g)** change the bacterial adhesion and biofilm formation through modification of the physicochemical properties of surfaces and bacteria. **(h)** Bacteriophages attach to the bacterial surface and **(i)** inject their genetic material inside the bacteria, **(j)** new bacteriophages DNA and proteins (including endolysins) are synthesized, resulting in bacteriophage assembly and multiplication inside the bacteria. **(k)** Endolysins act on bacterial lysis and membrane disruption, and bacteriophages are released being available to infect other bacteria. **(l)** Endolysins may act on biofilm control by disrupting EPS and biofilm structure. **(m)** EOs and phytochemicals also disrupt cell membrane and cause the leakage of intracellular content and **(n)** inhibit the metabolic activity and the energy production in exposed bacteria. In biofilms, **(o)** EOs and phytochemicals, due to their hydrophobic nature, may diffuse through EPS, **(p)** inhibit the cell communication systems (QS), and **(q)** reduce the expression of virulence. **(r)** NPs gradually release metal ions that disrupt the cell membrane and **(s)** generate ROS. NPs also **(t)** promote biofilm removal and **(u)** may prevent biofilm formation. Extracellular matrix-degrading enzymes only act on the biofilm **(v)** by degrading EPS, **(w)** releasing cells from the biofilm structure, and **(x)** disrupting the biofilm structure. Created with BioRender.com.

invading pathogens [21]. AMPs differ in molecular structure, amino acid composition, molecular weight, chemical stability, and antimicrobial effects. The main molecular features consist of a predominantly positive charge (from +2 to +9 net charge) and a high percentage of hydrophobic residues (40–50%) with polar and non-polar side chains asymmetrically distributed to adopt an amphiphilic structure [21]. Bacteriocins are a subgroup of AMPs that are produced by lactic acid bacteria with antimicrobial effects against other bacteria [22]. Nisin is a well-known and the most effective bacteriocin. It is approved by the United States (US) Food and Drug Administration (FDA) and is generally recognized as safe (GRAS status) for direct use in food to control pathogens [23].

The antimicrobial effects of AMPs can result from the selective interaction of positively charged AMPs with the negatively charged cell surface, inducing pore formation, followed by the leakage of intracellular components, and cell death [23]. Both Gram-negative and Gram-positive bacteria are negatively charged on their surface due to the presence of negative phospholipids (teichoic acid in Gram-positive bacteria and lipopolysaccharides in Gram-negative bacteria). However, diferences in cell envelope structures may affect the effectiveness of AMPs on their cytoplasmic membranes [24]. In Gram-positive bacteria, there is a cross-linked and thick peptidoglycan layer that should be penetrated by the AMP to reach the cytoplasmic membrane. In contrast, Gram-negative bacteria have an outer membrane, a thin layer of peptidoglycan, and an inner (cytoplasmic) membrane. Therefore, AMPs only will kill Gram-negative bacteria if they are able to disrupt both outer and cytoplasmic membranes. Therefore, some AMPs are very effective in the control of Gram-positive bacteria, however, the same performance is not observed for Gram-negative bacteria [24]. In terms of antibiofilm action, several authors demonstrated the potential of AMPs for biofilm inhibition (Table S1). Yasir et al. [25] reviewed the mechanisms involved in the interactions with biofilms and concluded that AMPs can disrupt the membrane potential in biofilm-embedded cells causing adenosine triphosphate (ATP) release, interrupting bacterial cell signaling systems through the downregulation of the transcription of QS systems, such as Las and RhI, degrade the EPS matrix (depending on the AMP, the action will be directed to the disintegration of polysaccharides, lipids, proteins, or even extracellular DNA), and downregulate genes responsible for biofilm formation such as by inhibiting genes controlling the mobility of extrachromosomal elements and transport and binding proteins. However, physicochemical interactions with EPS components may hinder AMP penetration, reducing the effective concentration reaching cells in the inner biofilm layers [22]. Owing to their better effects on biofilm inhibition rather than biofilm removal from surfaces, AMPs have been proposed as biopreservatives for the food processing industry. They are suitable for industrial applications since AMPs are stable under a wide range of temperatures, pH, presence of organic solvents, detergents, ethylenediamine tetraacetic acid (EDTA — a chelating agent), and salinity [26]. However, AMPs can be subjected to enzymatic degradation and resistance development at subinhibitory concentrations [22,26]. Another limitation is the high production cost. However, their gene-based origin makes peptide engineering practicable (genetically engineered bacteria), reducing production costs, and making AMPs potentially attractive for commercial purposes [27]. The recombinant production of AMPs has been scientifically explored to provide more specificity and effectiveness in the use of these molecules. In fact, through recombinant production, modifications in peptide sequence may be performed to increase AMP stability and antimicrobial action, and reduce AMP toxicity and production costs. However, AMP production through recombination is also challenging, since AMP toxicity may affect the growth of producing cells and can be easily degraded. Microcin J25 is an AMP with a strong inhibitory effect against Gram-negative bacteria and it has been produced recombinantly and tested *in vitro* against *Escherichia coli* and *Salmonella* [28]. Moreover, lactolisterin BU expressed in *Pichia pastoris* exhibited strong activity against Gram-positive and Gram-negative foodborne bacterial pathogens [29].

Apart from Nisin, which has been applied as a food preservative in more than 50 countries through the approval of the USA FDA, WHO, and European Food

Table 2

Mode of action, advantages, limitations, and main applications of antimicrobial strategies in the food industry.

Antimicrobial strategy	Mode of action	Applications in food industry	Main advantages	Main limitations
AMPs	Disruption of the cell membrane (broad-spectrum action); interruption of bacterial cell signaling system; degradation of EPS matrix; downregulation of genes responsible for biofilm formation	Direct use in food products (food additive); food packaging	High bioavailability (wide range of sources of AMPs); specificity against antibiotic-resistant bacteria (engineered AMPs); structure adaptability	May be more effective against Gram-positive than Gram- negative bacteria; effectiveness depends on pH and is affected by proteases' presence; high production costs
Biosurfactants	Destabilization of cell membranes; alteration of surface physicochemical properties reducing bacterial adhesion and biofilm formation	Direct use in food products (additive); cleaning and/or treatment of food-contacting surfaces	Environmentally friendly; low toxicity; low production cost; biodegradability; stability at extreme conditions	Lacks a comprehensive assessment of safety and risk analysis; high production cost in relation to chemical surfactants
Bacteriophages and endolysins	Specific target; cell lysis by targeting peptidoglycan structure in bacteria wall releasing new phages	Direct use in food products (additive); food packaging; cleaning of food-contacting surfaces	Reduced probability to cause horizontal gene transfer; environmentally friendly; high specificity; effectiveness against target bacteria; low toxicity to humans and animals; easy and low-cost production	A broad-spectrum action should be used as a cocktail; emergence of bacteriophage-resistant bacteria
EOs and other phytochemicals	Interaction with cell wall; leakage of intracellular components; inhibition of microbial metabolism; disruption of energy production; inhibition of QS	Direct use in food products (additive); food packaging; cleaning of food-contacting surfaces	GRAS; environmentally friendly; reduced selective pressure	Lower antimicrobial effectiveness than traditional biocides; low water solubility; high volatility
Extracellular matrix-degrading enzymes	Degradation of EPS matrix; disruption of biofilm integrity and cell release; high specificity	Cleaning of food-contacting surfaces	Environmentally friendly, high biodegradability; low toxicity	High cost; should be used in combination due to their specificity; chemically unstable; requires specific operational conditions (pH, temperature)
NPs	Gradual release of metal ions; destabilization of the cell membrane; ROS generation; inhibition of metabolism; mode of action still requires clarification	Direct use in food products (additive); food packaging; nanocoatings for food-contacting surfaces	Broad spectrum; low volatility; high-temperature stability	Toxicity of synthesis processes; potential for triggering mutations and horizontal gene transfer; potential genotoxicity and carcinogenicity; irreversible aggregation

Safety Agency, ε-polylysine and pediocin PA-1 have been used as food additives. ε-Polylysine was approved for direct use in foods in Japan, Korea, and the US. Pediocin PA-1 has been used as a preservative on ready-to-eat meat products, as approved by the FDA [30]. Many other AMPs have been studied as an alternative for incorporation in food or in the development of functionalized food packaging (Table S1).

Biosurfactants

Biosurfactants are naturally produced amphiphilic compounds (with hydrophilic and hydrophobic groups), which are an alternative to synthetic surfactants due to their environmentally friendly nature, high biodegradability, low toxicity, high specificity in the mechanism of action, and stability at extreme conditions (i.e. pH, temperature, and salinity) [31]. As membrane-active agents, biosurfactants can disrupt cell membrane integrity by the formation of pores and ion channels, causing leakage of intracellular components and cell death [32]. Additionally, biosurfactants can change surface physicochemical properties that directly affect cell adhesion and consequently biofilm formation [31]. Several studies reported the antibiofilm effects of biosurfactants with application in the food processing industry (Table S2). Despite antimicrobial and antibiofilm effects, the commercial use of biosurfactants is still limited mainly due to higher production costs compared with chemical surfactants. The challenge of reducing costs has been addressed through the optimization of operation conditions, selection of high-yield engineered strains, and use of low-cost raw materials or by-products as substrates [31].

Bacteriophages and endolysins

Bacteriophages (or phages) are viruses that can specifically target bacteria and cause cell lysis mediated by endolysins [33]. Several phage-based products are already approved by the US FDA to control foodborne pathogens, such as ListShield™, Salmo Fresh™, and EcoShield™, all from Intralytix Inc., USA. Among bacteriophages, lytic phages are the most attractive ones for disinfection, due to a reduced probability of horizontal gene transfer between bacteria [33]. Lytic phages support greater inhibition of biofilm formation compared with biofilm removal (Table S3). They can diffuse through the water pores or channels toward cells in deeper layers and/or produce phage-derived enzymes that disrupt the biofilm structure. The antimicrobial effects against mature biofilms were impaired by the physiological and metabolic state of biofilm cells and the presence of an EPS matrix. Metabolically active cells (in the biofilm surface or nutrient-rich conditions) are more susceptible to phage infection than slow-growing bacteria (in the inner layers or nutrient-limiting conditions) [34]. In addition, the target specificity limited the phage activity against mixed-species biofilms, in which sensitive bacteria can be protected by nonsensitive species that hindered phages from reaching the target cells. However, the presence of a second susceptible species (*Staphylococcus aureus* and *S. epidermidis*) resulted in enhanced phage activity [34]. Another limitation is the development of resistance that can increase the tolerance of bacterial hosts to phage activity. Bacteria–phage interactions provided reciprocal coevolution in the bacterial host (rapid resistance) and lytic phage (mutations in the receptors of cell surface), remaining a fraction of the bacterial population susceptible to the phage activity [35]. Thus, a mixture of phages (phage cocktail) is advantageous due to large broad antimicrobial effects and slower resistance development. Furthermore, their potential industrial application depends on the chemical interactions between phages and biocides (already used for disinfection). Agún et al. [36] demonstrated that phages did not affect bacterial susceptibility to biocides (i.e. benzalkonium chloride [BAC], triclosan, chlorhexidine, and hydrogen peroxide), while biocides influenced phage activity in bacterial growth inhibition. In general, the phage–biocide combination did not improve the disinfection efficacy against biofilms [36].

Endolysins are phage-encoded enzymes that target cell wall peptidoglycan, causing cell lysis [37]. They are an alternative to bacteriophages due to their antimicrobial activity independent of cell metabolic state and low probability of resistance development. The antibiofilm effects of endolysins were demonstrated by several authors (Table S3). The outer membrane of Gram-negative bacteria hurdles the access of endolysins to the peptidoglycan layer, making Gram-positive bacteria more susceptible to their action. To overcome this limitation, studies have been conducted to engineer or combine endolysins with outer membrane- permeabilizing agents, such as chelating agents, weak organic acids, AMPs, and physical stresses [38]. Recently, artilysins (outer membrane-penetrating endolysins) were developed through the fusion of an endolysin with a specific outer membrane-permeabilizing agent, becoming effective against Gram-negative bacteria [39].

Essential oils and other phytochemicals

EOs are a complex mixture of natural compounds synthesized by plants as secondary metabolites, also known as phytochemicals. Several EOs have GRAS status for human consumption, such as cardamom seed, cinnamon, citrus peels, clover, coriander, ginger, lavender, onion, sage, and thyme (substances GRAS: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=182). Diverse EOs and phytochemicals have a broad spectrum of action (against bacteria and fungi) and low resistance development. In general, their mode of action involves disruption of cell integrity with leakage of intracellular compounds, inhibition of metabolic activity, and disturbance in energy production, causing cell death [40]. When used against biofilms, their hydrophobic nature was proposed to enable their diffusion through the EPS matrix, reaching the inner colonizing cells. The antibiofilm effects of EOs/phytochemicals were reported by several authors (Table S4). EOs/phytochemicals were considered effective for biofilm

prevention; however, complete biofilm eradication or removal was not achieved, leading to subsequent biofilm regrowth. Additionally, several phytochemicals (and their synthetic derivatives) have been reported as QS inhibitors, reducing bacterial virulence and biofilm formation. Furthermore, QS inhibitors at subinhibitory concentrations did not affect cell growth and no selective pressure or resistance emerged [41].

The valorization of plant secondary metabolites as value-added metabolites (phytochemicals) active against pathogenic microorganisms in industrial settings is a sustainable strategy for a green economy transition. However, some drawbacks narrow their commercial use, such as low water solubility (hydrophobic nature) and high volatility that causes their release to the gas phase before reaching the target cells. Microemulsion and nanotechnology have been developed as effective delivery systems of phytochemicals against biofilms [42]. In addition, a single phytochemical treatment had much lower antimicrobial effectiveness than traditional antimicrobial agents, involving high doses to reach similar antimicrobial activity. For the food processing industry, synthetic phytochemical derivatives have been optimized to improve antimicrobial activity and QS inhibition effects, being exploited to be used as antioxidants to prevent lipid oxidation and extend the shelf life of food products [43].

Extracellular matrix-degrading enzymes

Enzymatic approaches have attracted attention for their ability to degrade the EPS matrix, which can disrupt the biofilm structure and promote the release of cells [44]. Extracellular matrix-degrading enzymes have the EPS matrix as a target in biofilm control, while endolysins target the peptidoglycan present in the bacterial cell wall. Many extracellular matrix-degrading enzymes, including proteases, lipases, cellulases, alginate lyases, polysaccharide depolymerases, dispersin B, and deoxyribonuclease (DNase I), are commonly included in cleaning agents, disinfectants, and detergents [44]. Recent studies on the use of extracellular matrix-degrading enzymes for biofilm control in the food processing industry are summarized in Table S5. Enzymatic treatment is typically more effective at inhibiting biofilm formation than removing mature biofilms [45]. Owing to their specificity, enzymes must be selected and combined based on the predominant composition of surface contaminants (i.e. organic load, proteins, eDNA, and polysaccharides) [46]. The composition and structure of the EPS matrix differ among microbial populations, resulting in different enzymatic requirements. Therefore, the EPS matrix composition should guide the design of enzyme-based biofilm cleaning strategies to be used in industrial settings [47]. Although enzymatic treatment has low antimicrobial effects, it can destabilize biofilm structure by disintegrating the EPS matrix and detaching biofilm cells. This allows antimicrobial agents to penetrate the biofilm structure and reach cells in deeper biofilm layers [44].

The action of extracellular matrix-degrading enzymes is highly influenced by the operating conditions, such as temperature, pH, and exposure time, which should be optimized to minimize enzyme concentration while improving the antibiofilm activity. However, despite their antibiofilm effectiveness in combination with other biocides, enzymatic application in industrial settings is still limited, partly due to high cost, chemical instability, and operational requirements (temperature, pH, and exposure time) compared with traditional strategies [48]. To overcome these limitations, enzyme immobilization on NPs can be employed, which would allow enzymes to be easily recovered after use and enable a more cost-effective application [49].

Nanoparticles

Nanotechnology is being used in the development of new antimicrobial agents using various nanomaterials, such as silver (Ag), copper oxide (CuO), and zinc oxide (ZnO). Several studies have demonstrated the effective and non-specific antimicrobial and antibiofilm activities of NPs (Table S6). Their mode of action is related to the gradual release of metal ions, which can generate reactive oxygen species (ROS) and inhibit cell growth, and to the adsorption of NP on the cell surface, which can mediate the disruption of cell integrity, change membrane permeability, and leakage of intracellular components, causing cell death [50]. Against biofilms, NPs demonstrated to inhibit their formation and promote removal.

The main drawbacks of NPs are associated with their inherently irreversible aggregation that can limit long-term applications, low metal ion release under oxygen depletion conditions, the potential for triggering mutations and horizontal gene transfer, and the harmful effects of their chemical synthesis (i.e. toxicity of the precursor materials and generation of hazardous by-products) [51]. To address these limitations, Haidari et al. [52] proposed the production and design of polycationic silver nanoclusters (pAgNCs) with a high cationic surface nature, ultrasmall size, and a high fraction of Ag ions. pAgNCs offer selective and controlled delivery of AgNPs with excellent antimicrobial activity in both aerobic and anaerobic conditions. As an alternative to conventional NPs, green-synthesized NPs, also known as biogenic NPs, from biological processes have several advantages, including a simple production process generally involving one step, no use of hazardous chemicals, and cost-effectiveness [51].

Combination of antimicrobial strategies

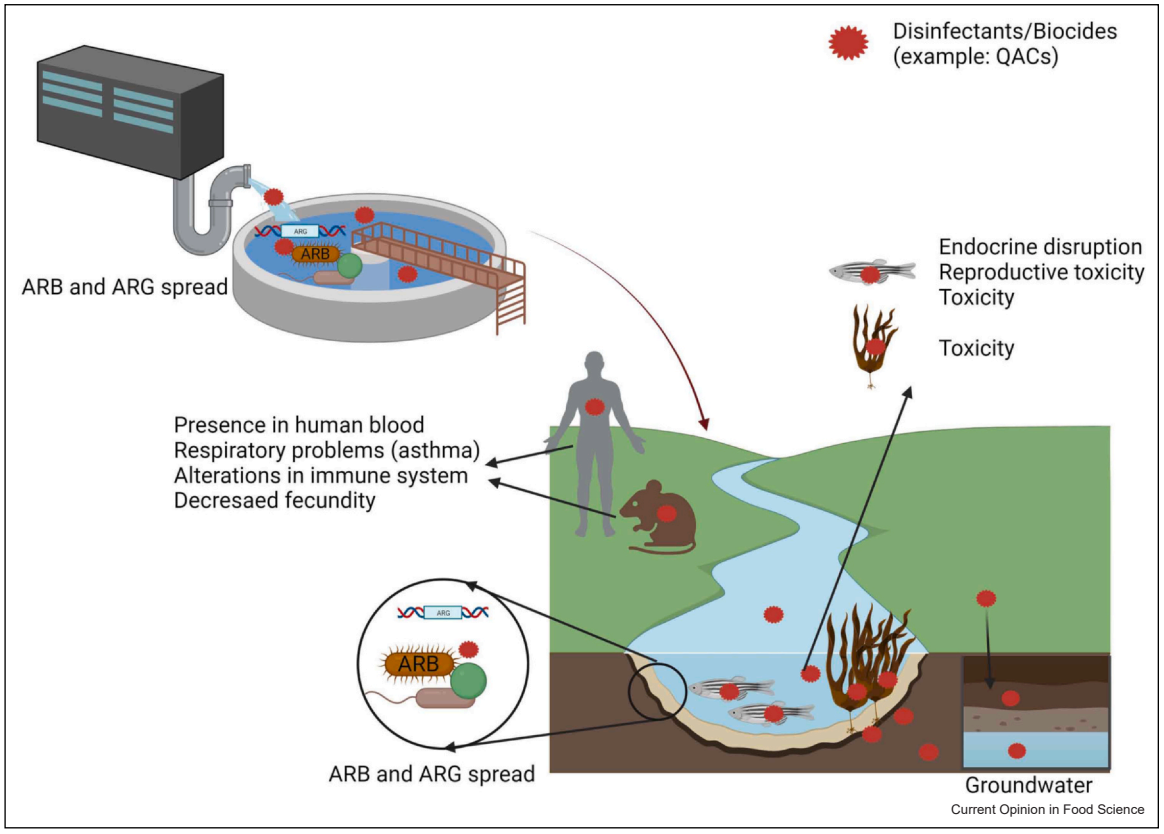
The current regulation on biocide registration, particularly in Europe, has effectively hindered most research into novel antimicrobial agents due to the high cost involved in the processes for development and approval. It is currently more economically viable to develop formulations containing approved active compounds than

to risk the cost of developing and attempting to gain authorization for novel antimicrobials [6]. Several reports have studied the antibiofilm effects of new antimicrobial strategies (Tables S1–S6). It is important to highlight that a single treatment cannot completely eradicate biofilms. Therefore, the use of working-safe, environmentally friendly, and cost-effective antimicrobial strategies, as well as combinations that lead to a synergistic effect, should be prioritized. Several combinations of two or more (non)-conventional strategies have shown to improve the antimicrobial efficacy for industrial applications, overcoming the biofilm tolerance to biocides. However, special attention should be given to the potential antagonistic effects and development of tolerance after combination exposure, which can lead to a subsequent increase in biofilm formation [53]. Several authors have demonstrated the potential synergistic/additive antibiofilm effects from combinations between different antimicrobial agents [5,54,55]. Fernandes et al. [5] showed synergistic/additive effects from a dual-biocide combination and triple combination of two biocides and a phytochemical (as QS inhibitor). Gómez et al. [54] proposed eugenol combined with a disinfectant solution as a promising strategy for biofilm control since synergistic effects were verified against *Pseudomonas* spp., *Enterococcus* spp., and *Staphylococcus* spp. Nutgall EO combined with a QAC promoted remarkable biofilm removal by allowing easy diffusion of EO toward cells in the inner layers of biofilm and QAC disruption of cross-linked hydrophobic interactions of EPS matrix components [55]. Other strategies include the combination of antimicrobial agents with physical treatments, such as ultrasonication, filtration, heat, steam, ultraviolet radiation, and automatic scrubbers [19].

Environmental impact and resistance development

The COVID-19 pandemic has significantly influenced the severity of microbial control procedures, leading to intensive high-level disinfection practices that may have negatively impacted the environment and increased the risk of antimicrobial resistance and cross-resistance [56]. Furthermore, biofilm control imposes critical biocide treatments that can be corrosive and potentially hazardous to humans and the environment. These treatments may result in a large release of chemicals difficult to remove in wastewater treatment plants, leading to environmental accumulation and

Figure 3



Dissemination of biocide residues in the environment and their impact in exposed biota. Created with BioRender.com. (ARG — antimicrobial resistance genes, ARB — antimicrobial-resistant bacteria).

promoting adverse ecological effects, such as harmful effects on aquatic organisms [57]. Several authors assessed and reviewed the environmental accumulation and risk of exposure to QACs [56,58,59]. However, limited data exist on the environmental impact of biocide release/accumulation. According to Fuchsman et al. [58], the estimated exposure to BAC, a QAC, in surface water, sediment, and soil, is lower than the applicable low-end toxicity values. However, QACs have high environmental persistence, low biodegradability rates, and high surface affinity [59]. Besides the potential toxicity, both biocide overuse (repeated exposure) and misuse (application of subinhibitory/sublethal concentrations) can result in the survival of exposed cells and the development of antimicrobial tolerance and cross-resistance to other biocides and antibiotics [60]. This selective pressure does not occur only in the food industry. The residual biocides discharged in wastewater (biocides at low concentrations) will maintain the selective pressure in the environment and increase the risk of microbial resistance development and spread. Figure 3 summarizes the possible dissemination of residues of biocides and their impact on the exposed biota. Attending to the concerning effects of conventional biocides on the environment, as well as for public health, the development and approval of eco-friendly alternatives urges. In fact, all the presented strategies for biofilm control have been described as more eco-friendly than traditional biocides. However, NPs have been controversial, although their efficiency on bacteria inactivation, their synthesis is not a sustainable process, and their release in the environment is also causing some concerns due to putative antibiotic resistance spread and nefarious consequences for exposed biota [51]. The combination of different strategies (conventional biocides with eco-friendly alternatives) may also be an important approach, not only to increase the effectiveness of biofilm control but also to reduce the in-use concentration of more environmentally concerning biocides.

Concluding remarks

Biofilm prevention and control in the food processing industry are an emergent challenge due to intrinsic biofilm tolerance mechanisms, which result in high economic losses and negative impacts on public health. The persistence of biofilms on surfaces, their potential for product contamination, and tolerance and cross-resistance events make the development of effective control strategies a priority. In recent years, scientific interest has been focused on environmentally friendly and cost-effective antimicrobial strategies (used either alone or in combination), and diverse studies have reported promising antibiofilm effects. The selection of strategies for biofilm control in food industries should take into consideration several aspects, such as food safety and quality, environmental consequences, and costs. Therefore, EOs and phytochemicals stand up by the reduced selective pressure imposed in the microbial community, in opposition to NPs that may promote horizontal gene transfer and whose production has toxic effects on the environment. Enzymes and AMPs will only be effective under controlled conditions (i.e. pH, temperature), therefore, their use in the food industry may be compromised and limited to specific processes. In terms of costs, the use of AMPs and enzymes may be the most expensive approach among those reviewed. The combination of conventional biocides with new approaches such as EOs or phytochemicals may have synergistic action on biofilm control while reducing the negative environmental impacts of the use of conventional biocides. Understanding the biofilm tolerance mechanism to biocides and developing effective disinfection strategies remains a research need. Addressing the challenges for effective and sustainable biofilm control will require collaborative efforts from the food processing industry, regulatory agencies, and the scientific community to ensure food safety and public health.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cofs.2024.101124.

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