



# Hurdle technology using encapsulated enzymes and essential oils to fight bacterial biofilms

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## Abstract

Biofilm formation on abiotic surfaces has become a major public health concern because of the serious problems they can cause in various fields. Biofilm cells are extremely resistant to stressful conditions, because of their complex structure impedes antimicrobial penetration to deep-seated cells. The increased resistance of biofilm to currently applied control strategies underscores the urgent need for new alternative and/or supplemental eradication approaches. The combination of two or more methods, known as Hurdle technology, offers an excellent option for the highly effective control of biofilms. In this perspective, the use of functional enzymes combined with biosourced antimicrobial such as essential oil (EO) is a promising alternative anti-biofilm approach. However, these natural antibiofilm agents can be damaged by severe environmental conditions and lose their activity. The microencapsulation of enzymes and EOs is a promising new technology for enhancing their stability and improving their biological activity. This review article highlights the problems related to biofilm in various fields, and the use of encapsulated enzymes with essential oils as antibiofilm agents.

## Key points

- *Problems associated with biofilms in the food and medical sectors and their subsequent risks on health and food quality.*
- *Hurdle technology using enzymes and essential oils is a promising strategy for an efficient biofilms control.*
- *The microencapsulation of enzymes and essential oils ensures their stability and improves their biological activities.*

**Keywords** Biofilm · Enzymes · Essential oils · Microencapsulation

## Introduction

Pathogenic bacterial contaminations of abiotic surfaces in food and medical sectors represent a serious public health problem, as they can lead to severe human infections worldwide (Abdallah et al. 2014; Khelissa et al. 2017). Foodborne infections generally occur after consumption of food and drink contaminated with pathogens. These contaminations

can occur during any step of food processing, through food handlers and contaminated food contact surfaces and equipment (Verraes et al. 2013). According to the annual report of the Centers for Disease Control and Prevention (CDC), 841 foodborne disease outbreaks were reported in the USA in 2017, resulting in 14,481 illnesses, 827 hospitalizations, 20 deaths, and 14 food product recalls (CDC 2019). The World Health Organization (WHO) reported that an estimated 600 million people – nearly one in ten people worldwide – become ill from consuming contaminated food and 420,000 die each year of which 30% occur among children under 5 years old. In addition, 110 billion US\$ are lost annually in medical expenses and productivity due to unsafe food in low- and middle-income countries (WHO 2020). In 2019, 1,783 foodborne illnesses were reported in France, affecting 15,641 people, of which 609 (4%) were hospitalized (hospitalization or emergency room visit) and 12 (0.08%) died (SPF 2021). Healthcare-associated infections

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(HCAs) are the most common adverse event in the healthcare field worldwide. These infections can occur in all types of healthcare settings through healthcare personnel's hands and contaminated devices and surfaces (catheters, surgical instruments, endoscopes, respiratory systems, needles, etc.) (Weber et al. 2013; Ssekitoaleko et al. 2020). According to the US Centers for Disease Control and Prevention, almost 1.7 million hospitalized patients contract HCAs each year while treated for other health problems, and more than 98,000 patients (one in 17) die from them (Klevens et al. 2007). In 2017, the National Point Prevalence Survey (PPS) on HCAI in France showed that one in twenty patients hospitalized in a healthcare facility were infected. The four main sites of HCAI, accounting for 71.5% of documented infectious sites – urinary tract infections (28.5%), surgical site infections (15.9%), pneumonia (15.6%), and bloodstream infections (11.4%) – were identical in 2012 and 2017 (SPF 2019).

In natural and artificial environments, bacteria tend to live attached to the abiotic surfaces and to develop a complex structure called biofilm. It has been found that approximately 40–80% of the bacterial cells on earth are able to form biofilms (Flemming and Wertz 2019). Biofilms, unlike planktonic cells, are a self-protected grown cluster of bacteria. They are defined as a structured microbial community, adhering to a surface, to interfaces and to each other, and embedded in a self-produced polymer matrix that offers a highly protective environment against biocide attack (Donlan and Costerton 2002; Karygianni et al. 2020). The formation of biofilms poses serious problems in many fields due to the potential increased resistance to chemical biocides, antibiotics, and UV radiation; increased secretion of secondary metabolic products; and high gene exchange rates (de Carvalho 2017; Xu et al. 2017; Gebreyohannes et al. 2019; Rodrigues and Černáková 2020). Several studies have shown that industrial ecosystems are favorable areas for bacterial growth and biofilm formation (Coughlan et al. 2016). In a hospital environment, biofilms have been found to survive and persist on many medical device surfaces and on the tissue of patients, resulting in several persistent infections (Dongari-Bagtzoglou 2008; Percival et al. 2015). Thus, the control of biofilms remains the most important task for many industries to reduce the microbiological risk associated with its persistence in these areas. Several strategies have recently been proposed to combat biofilms, which include chemical removal such as detergents, biocides, and surfactants; and mechanical removal such as thawing, freezing, sonication, and scraping (de Carvalho 2007; Zea et al. 2020). However, complete removal of biofilm by the single use of these methods has been shown to be difficult to achieve due to the high protection of biofilm cells by EPS that act as an initial protective barrier to the biofilm cells and make biofilm 10–1,000 times more resistant to antimicrobial

agents than the planktonic cells (Singh et al. 2017; Tan et al. 2018). In addition, although the sanitation is one of the most widely used and essential techniques to control biofilm in the industries, it is important to note that the application of these sanitizers for many decades could be a major cause of the emergence of antibiotic resistance in bacteria and their spread to pathogens, which has led to the search for new natural antimicrobial agents to overcome these issues (Bayoumi et al. 2012; al Kassaa et al. 2021).

The combined use of two or more hurdle methods to control biofilm (Hurdle technology) is a potentially effective strategy for an efficient biofilm cell removal from abiotic surfaces, as they would attack microorganisms in different ways (Khan et al. 2017). The synergistic effect of reducing bacterial contamination from abiotic surfaces using Hurdle technology has been successfully demonstrated in numerous studies (Lequette et al. 2010; Pechaud et al. 2012; Ban and Kang 2016; Lim et al. 2017, 2019; Jung et al. 2018; Husain et al. 2019). In this way, the combination of enzymes with bio-based antimicrobials will be a promising method for controlling biofilm in such a way that the enzymes would destabilize and destroy the biofilm matrix, so that bacteria protected by the matrix would be eliminated more effectively by the antimicrobials. It's now established that enzymes control and eliminate biofilms owing to their ability to degrade major components of the biofilm matrix, promote cell lysis, induce biofilm disruption, and disrupt cell-to-cell signals that govern biofilm maintenance and formation (Mohamed et al. 2018). Essential oils (EOs), which are aromatic oily liquids derived from plant materials are known as natural and safe bio-based biocides for synthetic drugs and antiseptics and have been widely tested *in vitro* against a broad range of pathogenic bacteria (Oulkheir et al. 2017). In addition to their antibacterial activity, numerous studies have demonstrated their anti-biofilm activity by removing and preventing biofilm formation (Vázquez-Sánchez et al. 2015; Oh et al. 2017; Engel et al. 2017; Mohamed et al. 2018). The use of EOs and their application have to face many challenges such as their volatility, stability issues, and their poor water solubility which may decrease their activity. It is therefore necessary to find out a new strategy, such as encapsulating these molecules in different material supports, to effectively improve their activity. Microencapsulation of EOs is a good tool to increase their stability, decrease their water immiscibility, control their release, and limit the physicochemical interactions between these molecules and the biofilm matrix components. These interactions are often associated with a decrease in the efficacy of the antibacterial molecules (Cui et al. 2016a; El Asbahani et al. 2015; Engel et al. 2017).

In this regard, this review will focus on the various factors that influence the adhesion of bacteria and the formation of biofilms on abiotic surfaces, as well as the biofilm resistance towards disinfectants. Moreover, the problems

associated with biofilms in food and medical sectors, and the different strategies for biofilm prevention and eradication will be highlighted, in addition to the effectiveness of hurdle technology in removing biofilms using different bio-based molecules. Finally, the place of the microencapsulation as a tool for the formulation of bio-based biocides to fight biofilm will be discussed.

## What is a bacterial biofilm and how is it formed?

Biofilms are generally defined as microbial populations that are irreversibly associated (cannot be removed by mild rinsing) with a surface and wrapped in a self-produced EPS matrix (Donlan 2002; Hall-Stoodley and Stoodley 2009). Microorganisms structured within biofilm differ from their planktonic counterparts by the transcribed genes. Bacteria are able to form biofilms when the growth conditions are suitable on different abiotic surfaces encountered in healthcare, food industries, industrial or potable water systems, and natural aquatic systems (Abdallah et al. 2014).

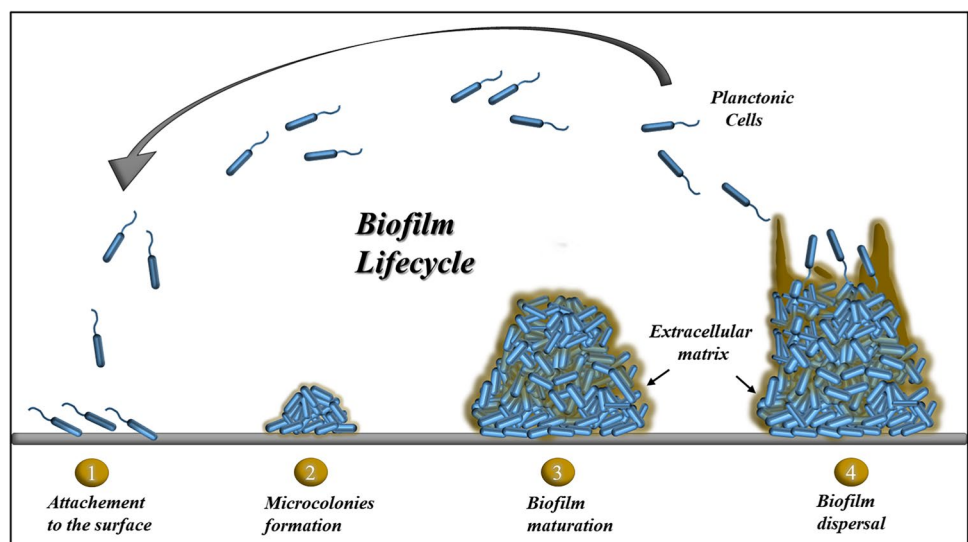
### Biofilm formation steps

The process of biofilm formation is complex and several factors may be involved. Bacterial cell adhesion and biofilm formation are significantly linked to the substratum properties such as hydrophobicity, electric charge, and rugosity. In addition, cell surface and the presence of pili, flagella, glycocalyx, or fimbriae are important for cell adhesion and biofilm formation (Donlan 2001; Roy et al. 2017; Hage et al. 2021). A bacterial biofilm can be structured in four common stages (Fig. 1). In the first stage, the bacterial cells bind to a biotic or abiotic surface (1); then the cells cluster

multiply and form microcolonies (2) followed by the formation mature biofilm (3). The last stage is the detachment and dispersion of bacterial cells in the surrounding environment (4) (Abdallah et al. 2014; Khelissa et al. 2017).

The initial stage is governed by the reversible interactions mediated by the non-specific Lifshitz-van der Waals, Lewis acid–base, and electrostatic forces (Kaplan 2010) and requires the presence of specific adhesins located on the host (e.g., fimbriae, flagella) (1) (Rosan and Lamont 2000; Abdallah et al. 2014). In the second step, the adherent bacteria synthesize exopolysaccharide proteins and other components of the polymer matrix that maintain the bacterial cells together in a mass and tightly fix the bacterial mass to the surface and contribute to the irreversible adhesion (2). In the third stage, the biofilm becomes mature and able to express different genes and contributes to the antimicrobial resistance of the biofilm (3) (Kaplan 2010; Mah and O'Toole 2001; Chakraborty and Kumar 2019). The final biofilm formation step is the dislocation of cells from the biofilm and their dispersion in the environment (4). Cell detachment can be triggered by a variety of factors such as mechanical disturbances, polymer matrix enzymatic degradation, surfactant production, and exopolysaccharide release (Kaplan 2010). These cells have the ability to adhere to new surfaces and re-form a biofilm and may contribute to biological dispersion, bacterial survival, and disease transmission that are known as biofilm lifecycle. As at other stages of biofilm development, bacteria respond to multiple environmental signals (e.g., nutrient concentrations), signal transduction pathways, effectors, and bacterial cell density, a phenomenon better known as quorum sensing (QS) (Karatan and Watnick 2009; Liu et al. 2019). QS is the regulation of gene expression in the response of cell density by the liberation of chemical signal molecules called autoinducers (acylated homoserine lactones as autoinducers of Gram-negative

**Fig. 1** Different stages of biofilm formation



bacteria and oligo-peptides as autoinducers of Gram-positive bacteria) that allows the differentiation of bacterial biofilm. When these molecules attain a minimal threshold stimulatory concentration, the activation or repression of new genes occurs (Zhao et al. 2020). Thus, QS allows bacteria to display many responses that benefit the population, such as enhanced accessibility to nutrients and more favorable environment and promotes action against competing bacteria and environmental stresses (Zhao et al. 2020).

### Extracellular polymeric substance, composition, and functions

A biofilm is composed of attached microbial cells encased within a matrix of EPS. EPS was initially designated as “extracellular polysaccharides,” but it has been renamed as “extracellular polymeric substance” because it can also contain many other substances (Flemming and Wingender 2010). EPS may represent 50 to 90% of the total organic carbon of biofilms (Evans 2014). The composition of the matrix varies according to the bacterial species, strains, and growing conditions; it is highly hydrated (can contain up to 97% water) and is mainly composed of proteins, polysaccharides, and extracellular DNA (eDNA) (Fulaz et al. 2019). Biofilm matrix can also contain surfactants, lipids, glycolipids, extracellular enzymes, and cations (Karatan and Watnick 2009; Flemming and Wingender 2010; Karygianni et al. 2020). Most of the today knowledge has been generated by using model organisms forming biofilms, in particular *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Streptococcus mutans*, and *Vibrio cholerae* (Fig. 2). Several advanced reviews have detailed the functional role and composition of EPS in various matrices formed by these organisms as biofilms of a single species (Zarnowski et al. 2014; Peterson et al. 2015; Hobley et al. 2015; Flemming et al. 2016; Dragoš and Kovács 2017; Bowen et al. 2018).

EPS, which has been termed as the “dark matter of biofilms,” may provide a potential number of challenges and play a very crucial role in adhesion, aggregation, cohesion, structural integrity, and protective barrier of biofilm (Fulaz et al. 2019). The EPS matrix provides key architectural and protective support for microbial communities in the biofilm, blocking access of xenobiotic and antimicrobials to biofilm cells and providing protection against environmental stresses such as pH change, UV radiation, desiccation, and osmotic shock (Al Kassaa et al. 2019; Karygianni et al. 2020).

The polysaccharides play a fundamental role in the biofilm formation and antibiotic tolerance and as a virulence factor in opportunistic pathogens. Examples of the most common polysaccharides are alginate, cellulose, polysaccharide synthesis locus (PSL), and pellicle (PEL)

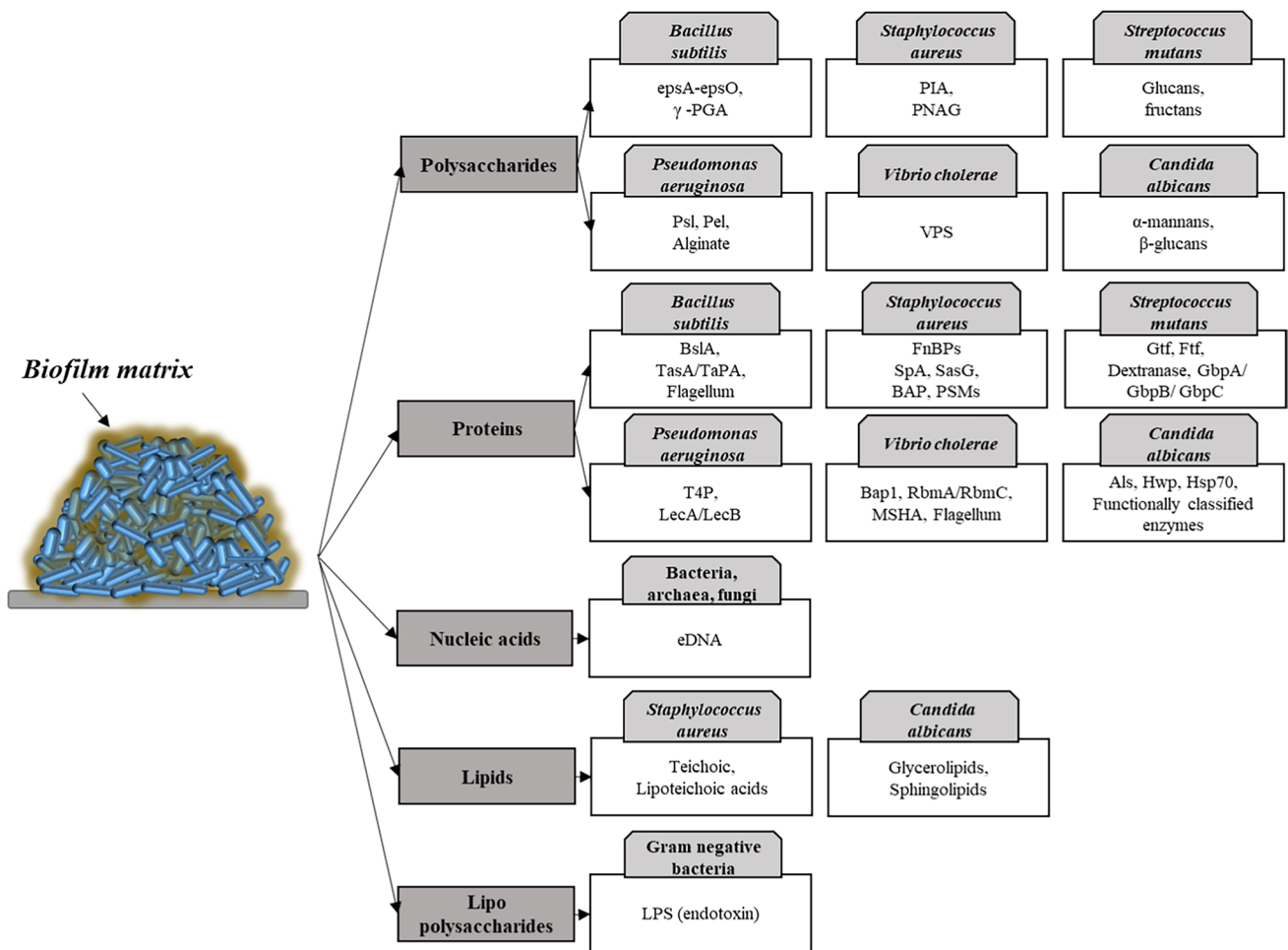
polysaccharides Pel, Psl, and the staphylococcal polysaccharide intercellular adhesin (PIA) (Fulaz et al. 2019; Bundalovic-Torma et al. 2020). The proteins play also an essential role in biofilm adhesion and cohesion and, in some cases, are found in higher proportions than polysaccharides (Karygianni et al. 2020). The common proteins presented in the biofilm matrix are amyloid fibers (Fulaz et al. 2019). Furthermore, previous studies have shown that eDNA plays a significant role in the structural stability, formation, and integrity of the bacterial biofilms (Devaraj et al. 2019) (Table 1).

### Biofilm architecture

Although some structural properties of biofilm can generally be regarded as universal, it has been reported that each biofilm community is unique (Tolker-Nielsen and Molin 2000). The morphology of biofilm can be rough smooth and flat or filamentous; furthermore, the biofilm can change on its degree of porosity, with mushroom-like macrocolonies surrounded by voids filled with water (Flemming and Wingender 2010). The concept of this diversity of structure is descriptive not only for mixed crop biofilms (environmental biofilms) but also for pure crop biofilms common to medical devices and those associated with infectious diseases (Donlan 2002). Several parameters can explain this heterogeneity, including the surface properties (e.g., hydrophobicity, roughness, electrochemical properties), hydrodynamic forces (e.g., mass transfer, shear forces, frictional drag, form drag), the presence of nutrients or inhibitors (e.g., concentration, antimicrobial properties, mass transfer properties, reactivity), and the consortia and ecological diversity of the biofilm (e.g., cell signal, presence of morphotypes, motility, food chains, trophic structure) (Stoodley et al. 1997). In addition, the structured communities of biofilm depend highly on the quantity, characteristic, and the three-dimensional structure (the dense areas, pores, and channels) of the EPS (Sutherland 2001).

### Factors influencing bacterial cells adhesion

The attachment of a cell to a substrate is called adhesion, and the attachment of one cell to another is called cohesion (Garrett et al. 2008). For the first steps of adhesion, the interactions between the conditional layer and the substrate strongly influence the growth of cellular communities; this layer can be composed of many organic or inorganic particles and modifies the substrates which facilitate the accessibility to bacteria. The biofilm adheres in a reversible or irreversible manner. Factors such as available energy, surface functionality, bacterial orientation, temperature, and



**Fig. 2** Composition of extracellular polymeric substances (EPS) in the biofilms of some model organisms, epsA-epsO operon-encoded exopolysaccharide, poly- $\gamma$ -glutamate ( $\gamma$ -PGA), polysaccharide intercellular adhesin (PIA), poly- $\beta$ (1–6)-N-acetylglucosamine (PNAG), *Vibrio cholerae* *Vibrio* polysaccharide (VPS), biofilm surface layer protein (BslA), translocation-dependent antimicrobial spore component (TasA)/TasA anchoring and assembly protein (TapA), fibronectin-binding proteins (FnBPs), staphylococcal protein A (SpA), *Staphylococcus aureus* surface protein G (SasG), biofilm-associated protein (BAP) extracellular, phenol-soluble modulins (PSMs), glucosyltransferases (Gtf), fructosyltransferases (Ftf), glucan binding

proteins (GbpA, GbpB, GbpC), type IV pili (T4P), lectins (LecA/LecB), biofilm-associated protein (Bap1), rugosity and biofilm modulators (RbmA/RbmC), mannose-sensitive hemagglutinin (MSHA) pili, agglutinin-like sequence protein (Als), hyphal wall proteins (Hwp) cell wall, heat-shock proteins (Hsp70). Further details on EPS components, including those from other microbes (such as *Escherichia coli*), are available from the following references (Mann and Wozniak 2012; Vlamakis et al. 2013; Zarnowski et al. 2014; Teschler et al. 2015; Hobley et al. 2015; Ibáñez de Aldecoa et al. 2017; Dragoš and Kovács 2017; Cochet and Peri 2017; Bowen et al. 2018; Nett and Andes 2020)

pressure conditions have a significant effect on the initial adhesion of bacterial cells. Then if the repellent forces are higher than the attraction forces, the bacteria detach from the surface; this is more apt to occur before a substrate is conditioned (Garrett et al. 2008). If the physical appendages of bacteria (flagella, fimbriae, and pili) overcome the repellent physical forces of the electric double layer, a number of reversibly adsorbed cells remain immobilized and become irreversibly adsorbed (Weger et al. 1987). Some research has shown that microbial adhesion is highly dependent on the hydrophobic-hydrophilic and topography properties of interacting surfaces (Liu et al. 2004; Hage et al. 2021).

**Factors of biofilm resistance**

There are different factors related to the physiological and structural characteristics of a biofilm that influence its resistance to disinfectants. Biofilm can be protected against antimicrobials by the limitation of diffusion or reaction of disinfectants; thus, due to the presence of EPS and a multiple layer of cells that can form a complex and dense structure, biocides have a difficult entering and achieving the inner layers, which affects their effectiveness (Bridier et al. 2011). The organic matter present in the matrix such as proteins, nucleic acids, or carbohydrates can deeply interfere with

**Table 1** The role of EPS in biofilm formation

Function	Relevance for biofilm	EPS components involved	References
Adhesion	Allows the first steps in the colonization of abiotic and biotic surfaces by planktonic cells and the long-term fixation of entire biofilms to the surfaces	Polysaccharides, proteins, DNA, and amphiphilic molecules	(Flemming et al. 2007)
Aggregation of bacterial cells	Enable binding between bacterial cells and biofilm cohesion that allows bridge construction between cells, the increase in cell density, the overall mechanical stability of biofilm and the determination of the biofilm architecture	Polysaccharides, proteins, and DNA	(Dunne 2002)
Cohesion of biofilms	Formation of the biofilm matrix, ensuring the mechanical stability of biofilms, determining the architecture of the biofilm, and allowing communication between cells	Charged and neutral polysaccharides, proteins (such as lectins and amyloids), and DNA	(Karygianni et al. 2020)
Retention of water	Retains a highly hydrated microenvironment around the biofilm which contributes to their tolerance to desiccation in water-poor environments	Hydrophilic polysaccharides and possibly, proteins	(Flemming 2016)
Protective barrier	Defensive barrier by providing resistance to defenses during infection by host or non-specific defenses and provides tolerability to various antimicrobial agents as well as environmental stress	Polysaccharides and proteins	(Leid 2009)
Sorption of organic and inorganic substances	Promotes the accumulation of nutrients from the environment, the sorption of xenobiotics, the formation of polysaccharide gels, the exchange of ion, the formation of minerals, and the accumulation of metallic toxic ions (thus contributing to the detoxification of the environment)	Charged or hydrophobic polysaccharides and proteins	(Fulaz et al. 2019)
Enzymatic activity	Allows the sequestration of dissolved and particulate substances in the environment, which provide nutrients to biofilm organisms and the degradation of the matrix EPS which permits the liberation of cells from biofilms	Proteins	(Flemming et al. 2007; Dewasthale et al. 2018)
Nutrient source	Offers a source carbon, nitrogen, and phosphorus for consumption by the biofilm cells	Potentially all EPS components	(Flemming and Wingender 2010)
Exchanging of genetic material information	Promotes cross-gene transfer between biofilm cells	DNA	(Madsen et al. 2012)
Redox activity	Play an important role in extracellular electron transfer reactions	Proteins (e.g., those forming nanowires and pili) and possibly, humic substances	(Cao et al. 2011)
Over-energy storage	Storage of excess carbon	Polysaccharides	(Bester et al. 2011)
Binding of enzymes	Accumulation, retention, and stabilization of enzymes by their interaction with polysaccharides	Polysaccharides and enzymes	(Lens et al. 2003)

the efficiency of disinfectants (Banach et al. 2015). The phenotypic adaptation of biofilm cells to nonlethal doses of disinfectants can also lead to biofilm resistance toward biocides. In addition, due to the limited penetration of antimicrobials and the low levels of exposition of the biofilm deep layers to the antimicrobial agent, the biofilm can develop adaptive responses to sublethal concentrations of the disinfectant (Bridier et al. 2011). Moreover, the physiological adaptations of biofilm cells, such as the expression of specific genes according to the environmental conditions of biofilms, allow the increase of the biofilm resistance. Many studies confirmed this adaptation by the comparison of gene expression profiles, and proteomic analyses of planktonic and biofilm states in different species (Sauer 2003; Whiteley et al. 2001; Abdallah et al. 2014), such as the expression of specific genes encoding for changes in membrane composition (Wolska et al. 2016), efflux pump (Soto 2013), and enzyme production. It has been reported that in *Pseudomonas aeruginosa* biofilms, quorum sensing is involved in the expression of catalase and superoxide dismutase genes. These enzymes are involved in protection against oxidative stress (Ahmed et al. 2019). Biofilm can also develop resistance by mutations and gene transfers (plasmids, transposons, or integrons) that provide cell-specific characteristic such as metabolic capabilities, virulence expression, and antimicrobial resistance (Bridier et al. 2011). Moreover, multispecies biofilms can protect against antimicrobials when present in complex communities, where interactions between species can lead to the formation of a large complex matrix, protection of members of bacterial communities, and expanded gene pool with more efficient passive resistance, quorum sensing systems, DNA sharing, metabolic cooperation, and many other synergies (Elias and Banin 2012; Wolcott et al. 2013). For instance, in vitro studies conducted on polymicrobial biofilms including *Staphylococcus epidermidis* and *Candida albicans* showed an altered susceptibility of each species to antimicrobial drugs due to their mutual interactions: the EPS of *Staphylococcus epidermidis* inhibited the penetration of fluconazole, while *Candida albicans* appeared to protect *Staphylococcus epidermidis* from vancomycin (Adam et al. 2002).

### Microbiological hazard associated with bacterial biofilms

Bacteria are capable of colonizing and forming biofilms on almost any type of surface, including synthetic and natural surfaces (Hall-Stoodley et al. 2004; Sweet et al. 2011). Bacterial structured biofilms improve the ability of bacteria to survive under stress and cause serious problems in many sectors such as industries, water systems, medical facilities, and public health (Khelissa et al. 2017; Jamal et al. 2018; Di Pippo et al. 2018; Avila-Novoa et al. 2018). The

detrimental effects of biofilms on the human society are therefore multiple.

The high capacity of bacteria to adhere and form biofilms on abiotic surfaces is a main concern for industries that provide a suitable environment for their formation (Donlan 2002; Simões et al. 2010; Flemming et al. 2013). Biofilms provoke biofouling of the industrial equipment such as cooling towers and heat exchangers. The biofouling is defined operationally as the development of biofilm that exceeds a defined threshold of interference (Murthy and Venkatesan 2009). This problem leads to energy loss, effective heat transfer, increased fluid friction resistance, and accelerated corrosion, as well as reduced the quality of product and many process additives and chemicals (Xiong and Liu 2010).

For example, the formation of biofilm in water distribution systems leads to reduced water quality and increased health risks (Dewanti and Wong 1995; Rao et al. 1998; Barak 2006). In the paper industry, biofilms cause the breakdown of chemicals such as calcium carbonate sludge and starch that are added to pulp sludge in the wet-end processing (Barak 2006).

In addition, the presence of biofilms is widespread in the food industry. They can be present on all kinds of surfaces such as plastics, glass, metal, wood, and food products (Chmielewski and Frank 2003). Microbial cell adherence to food contact surfaces is a serious concern for the food service and food processing industries, as adhesion can lead to cell survival and biofilm growth, allowing cross- and post-processing contamination. This reduces the shelf life of food products and constitutes the major factor of foodborne diseases (Shi and Zhu 2009; Bridier et al. 2015). In general, abiotic surfaces in contact with product may be cleaned many times per day, whereas environmental surfaces like walls may be cleaned weekly. This provides a longer time for adherent cells to grow on environmental supports. Thus, the extensive colonization of surfaces and the formation of mature biofilm may occur on these environmental surfaces. However, most of food product contact surfaces can hold only the adherent bacteria cells and young biofilm (Gibson et al. 1999). These adherent cells and biofilms not only pose a hygiene hazard in the food industry but also contribute to the economical costs due to technological failures, impedance of heat transfer, mechanical blockage, and metal surfaces corrosion (Houdt and Michiels 2010; Téllez 2010). Thereby, the need for efficient cleaning techniques is necessary to prevent the hazardous and expensive damage that bacterial biofilms can cause (Chmielewski and Frank 2003). Although many species of bacteria are able to form biofilms in the food industry, among the major genera of foodborne bacteria that are biofilm producers are *Pseudomonas*, *Listeria*, *Enterobacter*, *Flavobacterium*,

*Alcaligenes*, *Staphylococcus*, and *Bacillus* (Télez 2010). Most importantly, *Pseudomonas* contributes to the formation of polymicrobial biofilms with other foodborne pathogens providing them shelter for persistence (Bai et al. 2021).

In healthcare environments, biofilms can be found on several biomedical device surfaces (e.g., pacemakers, catheters, prosthetic heart valves, contact lenses, breast implants, and cerebrospinal fluid shunts) and on patient's tissues (dead tissues, e.g., bone sequestration and living tissues, e.g., tooth surfaces, lung tissue) (Hall-Stoodley et al. 2004; Wu et al. 2015; Alav et al. 2018). Several Gram-negative and Gram-positive bacteria can form biofilms on the biomedical devices, but the most commonly found are *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Enterococcus faecalis*. It has been reported that approximately two-thirds of infections related to medical devices are attributed to *staphylococcal* species (Hall-Stoodley et al. 2004; Shokouhfard et al. 2015; Pakharukova et al. 2018; Khatoon et al. 2018). *Pseudomonas aeruginosa* can also form biofilms on the interior surfaces in hospital water distribution systems (Loveday et al. 2014). In addition, infections caused by enterococci have become of particular concern in recent years because of their ability to develop resistance against a wide range of antimicrobial drugs used in medical practice. They are also involved in serious life-threatening infections in patients suffering from cancers or chronic diseases (Boccella et al. 2021). Furthermore, the emergence of polymicrobial infections has serious implications for patient care because of the difficulties associated with selecting the most appropriate antimicrobial therapy, particularly when multidrug-resistant pathogens are implicated (Francolini and Donelli 2010). Bacteria forming biofilms can cause several life-threatening human diseases and infections such as otitis media, infective endocarditis, osteomyelitis, periodontitis, cystic fibrosis, and chronic wounds (Southey-Pillig et al. 2005; Akyıldız et al. 2013; Masters et al. 2019). It has been reported that biofilm is involved for more than 65% of all microbial infections and has a high resistance to antimicrobials and host defense system components (Jamal et al. 2018; Ciofu and Tolker-Nielsen 2019). Hence, biofilms have a considerable impact on the human healthcare.

## Biofilm control

The controlling of biofilm accumulation remains the most arduous task for the many industries for which it is very important that both the inactivation and removal of biofilms from surfaces have to be realized (Simões et al. 2003; Dzia-nach et al. 2019).

As discussed previously, bacteria structured in biofilms are more resistant, than planktonic cells, to physical and

chemical methods used in cleaning and disinfection of abiotic surfaces (Martin and Feng 2009). Several methods and strategies can be used to control biofilm such as chemical treatment, mechanical removal, quorum sensing inhibition, nanotechnological method, enzymatic dispersion, bio-surfactants, and biosourced compounds such as essential oils derived from plants, bacteriocins, and bacteriophages (Fig. 3).

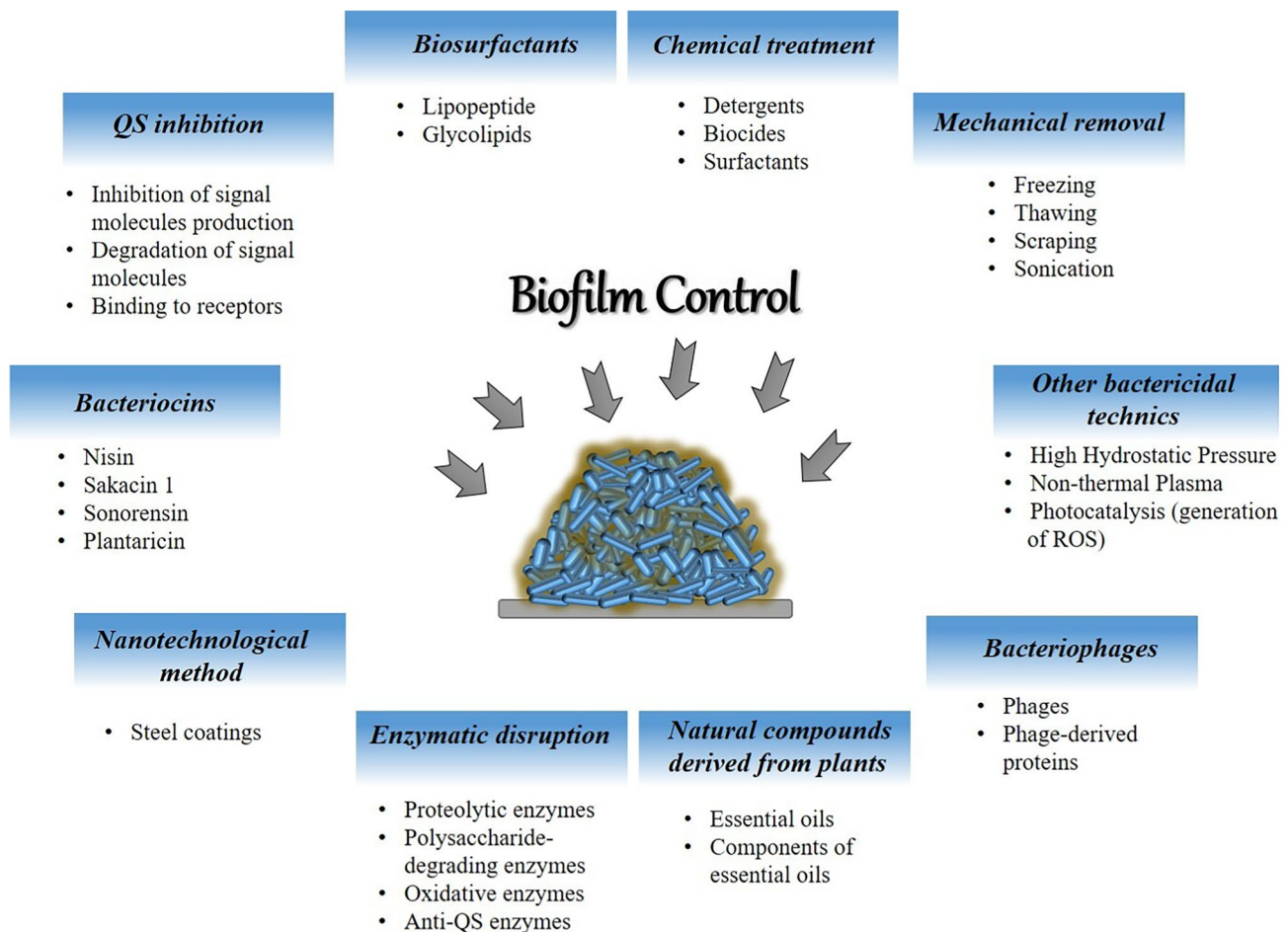
It is necessary to first understand the difference between disinfectants and sanitizers used in the industry. Disinfection means irreversibly destroying or inactivating specific infectious fungi and bacteria, but not necessarily spores, on hard surfaces. However, sanitizing means reducing microorganisms to levels considered safe for humans (Allan Pfuntner 2012).

Several chemical disinfectants can be used to treat biofilms such as NaOCl, peracetic acid, NaOH, and H<sub>2</sub>O<sub>2</sub>. The efficiency of these disinfectants is related to their oxidation of cellular structures (Rosenberg et al. 2008; Bayoumi et al. 2012; Nam et al. 2014; Bang et al. 2014; Ban and Kang 2016; Møretro et al. 2017; Yang et al. 2017; Alvarez-Ordóñez et al. 2019). However, previous studies have indicated that most of these disinfectants have little or no significant effect on the removal of established biofilms (Walker et al. 2007). It has been reported that disinfection with chlorine and chlorine dioxide can decrease the concentration of planktonic cells, but has no effect on biofilm biomass (Berry et al. 2006). Other studies show that treatment with sodium hypochlorite, the main commercial disinfectant does not significantly reduce the biomass of biofilms formed by *Escherichia coli* on the stainless steel surface (Lim et al. 2019). Chlorine is known as the most widespread artificial chemical disinfectant used because of its broad antimicrobial spectrum, easiness of application, and cost-effectiveness. Nevertheless, it is rapidly inactivated by organic matter. Moreover, chlorine activity is pH dependent and exhibits corrosion even to stainless steel and may combine with organic compounds to form toxic by-products (Chmielewski and Frank 2003; Guzel-Seydim et al. 2004; Houdt and Michiels 2010). In addition, it is important to note that the use of these sanitizers for decades could be one of the main causes of the development of antibiotic resistance in bacteria and their spread to pathogens (Capita and Alonso-Calleja 2013). These issues combined with the growing consumer concerns about their own health and environmental consciousness are leading to setup new alternative strategies to control biofilm such as the use of biosourced active molecules such as biosourced enzymes and essential oils (Knowles et al. 2005; Desai et al. 2012).

## Enzymatic disruption

The use of enzymes is an effective tool for eradicating biofilm owing to its ability to degrade the physical integrity





**Fig. 3** Different methods for biofilm control, quorum sensing (QS), and reactive oxygen species (ROS) (de Carvalho 2007; García-Almendárez et al. 2008; Winkelströter et al. 2011, 2015; Torres et al. 2011; Bayoumi et al. 2012; Beyth et al. 2015; Silva 2015; Nobrega

et al. 2015; Chopra et al. 2015; Scholtz et al. 2015; Coughlan et al. 2016; Gutiérrez et al. 2016; Coronel-León et al. 2016; Campana et al. 2017; Nica et al. 2017; Castellano et al. 2017)

of the EPS by binding and breaking down the components of the EPS into smaller units that can be transferred across cell membranes and then metabolized, thus destroying the multi-structural biofilm (Xavier et al. 2005; Mohamed et al. 2018). By a pre-treatment using enzymes, the biocides can be substituted or their concentration can be considerably reduced since the enzymatic effect on the EPS matrix promotes the access of the chemical biocides to the cells (Meireles et al. 2016). A wide range of enzyme applications have been described (Table 2) with the aim of reducing microbiological biofilm risk and replacing hazardous and ineffective chemical biocides, as well as providing an alternative green solution against biofilm formation due to their high biodegradability and low toxicity (Cortés et al. 2011; Srey et al. 2013). These characteristics make enzymes as a high-performance tool for controlling biofilm; thus, they are commonly used in detergents used in many industries (Torres et al. 2011; Huang et al. 2014).

Nevertheless, the enzyme effectiveness in eradicating and destroying biofilm is highly dependent on the composition of the matrix (Walker et al. 2007). Due to the heterogeneous composition of this matrix, different types of enzymes can be used to destroy biofilms. These enzymes can be applied individually or in combination with a complementary treatment (Meireles et al. 2016). There are currently four types of enzymes of potential interest in biofilm removal: polysaccharide degrading enzymes, proteolytic enzymes, anti-QS, and oxidizing enzymes that belong to three main classes, hydrolase, lyases, and oxydoreductases (Boels 2011; Thallinger et al. 2013; Huang et al. 2014; Coughlan et al. 2016; Meireles et al. 2016).

### Essential oils (EOs) as antibiofilm compounds

Essential oils (EOs) are volatile and aromatic liquids derived from plants. These compounds can be composed of complex

**Table 2** Examples of application of enzymes as anti-biofilm, their classification, and targets

Types of enzyme	Enzymes applied	Target biofilm	Results	References
Proteolytic enzymes	Savinase	<i>Pseudomonas fluorescens</i>	> 75% biofilm removal	(Molobela et al. 2010)
	Endolysin (LysH5)	<i>Staphylococcus aureus</i>	1–3 log biofilm removal	(Gutiérrez et al. 2014)
	Bromelain	<i>Klebsiella pneumonia</i>	74.6% biofilm removal	(Mohamed et al. 2018)
	Savinase, everlase	<i>Pseudomonas fluorescens</i>	> 80% biofilm removal	(Molobela et al. 2010)
	Esperase		74% biofilm removal	
	Mixed protease		75% biofilm removal	
	Savinase	<i>Pseudoalteromonas</i>	Total biofilm removal	(Leroy et al. 2008)
	Protease P4	Seven types of biofilm	> 70% biofilm removal	(Lequette et al. 2010)
	Protease P2	Three types of biofilm		
	Papain P1	Eight types of biofilm		
	Papain P3	Nine types of biofilm		
	Proteinase K	<i>Staphylococcus lentus</i>	Strongly removing of biofilm	(Fagerlund et al. 2016)
	Trypsin	<i>Staphylococcus cohnii</i> <i>Staphylococcus saprophyticus</i>		
	Dispersin B	<i>Staphylococcus epidermidis</i> <i>Staphylococcus aureus</i>	Strongly removing of biofilm	(Fagerlund et al. 2016)
	Trypsin	<i>Pseudomonas aeruginosa</i>	Strongly destroying biofilm	(Banar et al. 2016)
	Proteinase K	<i>Escherichia coli</i>	91.1–99.5% biofilm inhibition	(Lim et al. 2019)
	Polysaccharide-degrading enzyme	$\alpha$ -Amylase	<i>Staphylococcus aureus</i>	79% biofilm removal
Dispersin B		<i>Staphylococcus epidermidis</i>	40% biofilm removal	(Brindle et al. 2011)
Fungamyl		<i>Pseudomonas fluorescens</i>	> 80% biofilm removal	(Molobela et al. 2010)
Amyloglucosidase			> 50% biofilm removal	
Mixed amylases			> 70% biofilm removal	
Amylase S1		Six types of biofilms	Biofilm removal	(Lequette et al. 2010)
Polysaccharidase mix A		Three types of biofilms		
$\alpha$ -mannosidase, $\beta$ -mannosidase		<i>Pseudomonas aeruginosa</i>	Strongly destroying biofilm	(Banar et al., 2016)
Pectin esterase		<i>Pseudomonas fluorescens</i>	Three quarters of the biofilm cells	(Orgaz et al. 2007)
Oxidative enzymes	Cellulase	<i>Escherichia coli</i>	65.5–98.5% biofilm inhibition	(Lim et al. 2019)
	DNase	<i>Listeria monocytogenes</i>	50% biofilm removal	(Nguyen and Burrows 2014)
Anti-QS enzymes	DNase I	Gram-negative and Gram-positive biofilm	Biofilm removal	(Tasia et al. 2020)
	Lactonase	<i>Pseudomonas aeruginosa</i>	> 70% biofilm removal	(Kiran et al. 2011)
	acylase	<i>Pseudomonas aeruginosa</i>	60% biofilm inhibition	(Grover et al. 2016)

mixtures and of low-weight molecules, whose typical main components are dependent on the plant source (Engel et al. 2017). The biological activities of EOs and their components are largely recognized, including antimicrobial activities against bacteria, yeasts, and molds (Burt 2004; Reyes-Jurado et al. 2015; Calo et al. 2015).

EOs or their purified antimicrobial components are natural alternative biocides that have recently attracted attention as potential cleaners for the following reasons. (i) Many studies suggest that the chemical antimicrobial agents, currently used, trigger the development of antimicrobial resistance in the target microorganisms (Boakye et al. 2019). However, the development of bacterial resistance to EOs is limited because each EO is composed of a mixture of

various active antimicrobial agents (Wińska et al. 2019). (ii) The vapors emitted by EOs are highly bactericidal that can offer an additional advantage for the disinfection of hard-to-reach areas that need to be cleaned (López et al. 2005, 2007). (iii) Due to the ongoing trend towards green technology and changing consumer attitudes, there is a commercial advantage in antimicrobial agents that can be classified as “green,” such as EOs of plant origin (Soni et al. 2013).

The antimicrobial activity of EOs is caused mainly by their hydrophobic characteristic, which helps them to disperse into bacterial cell membrane lipids, causing disruption of the structure and increasing its permeability. This can lead to leakage of ions and other cell molecules and then lead to cell death (Rao et al. 2019). In general, EOs are

slightly more effective against Gram-positive bacteria than Gram-negative ones (Ratledge and Wilkinson 1988; Davidson and Naidu 2000; Canillac and Mourey 2001; Cimanga et al. 2002; Delaquis et al. 2002). Gram-negative bacteria can be expected to be less sensitive to the action of EOs since they possess a lipopolysaccharide-coated outer membrane that surrounds the cell wall and limits the diffusion of hydrophobic compounds (Ratledge and Wilkinson 1988; Burt 2004). Nevertheless, not all research on antimicrobial activity of EOs has shown that Gram-positive bacteria are more susceptible (Wilkinson et al. 2003). Furthermore, the antimicrobial activity of EOs is linked to their interactions, chemical composition, and the volatile molecules proportions (Dhifi et al. 2016).

EOs extracted from different plants can be used for their antibacterial effect such as monoterpenoids (such as borneol, camphor, carvacrol, eucalyptol, limonene, pinene, thujone), sesquiterpenoids (such as caryophyllene, humulene), and flavonoids (such as cinnamaldehyde and other phenolic acids) (Campana et al. 2017). In general, EOs with a high level of phenolic compounds, such as eugenol, carvacrol, and thymol, have significant antibacterial activities. These components are primarily responsible for disruption of the cytoplasmic membrane, electron flow, active transport, proton motive force, and coagulation of the cell contents (Dhifi et al. 2016). Numerous studies prove the antimicrobial activity of EOs against one or more microorganisms (Sivropoulou et al. 1996; Lambert and Johnston 2001; Ooi et al. 2006; Rota et al. 2008; Xu et al. 2008). Moreover, EOs have been shown to be highly effective against the most serious foodborne pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. (Braga et al. 2006). In addition, several studies (Table 3) demonstrate that essential oils have a significant antimicrobial activity against biofilm and biofilm formation (Soni et al. 2013; Neyret et al. 2014; Amaral et al. 2015).

### Hurdle technology as an efficient strategy to control biofilms

Due to the complexity of biofilms, a single use of a disinfectant may be insufficient to remove the entire undesirable biofilm. Hurdle technology involves the combined intelligent use of hurdles such as physical–chemical, chemical–chemical, or biological–chemical disinfection methods to achieve effective control of undesirable monomicrobial and polymicrobial biofilms by striking different targets within bacterial cells at the same time (Fig. 4) (Yuan et al. 2021). The synergistic effect of hurdle technology in reducing biofilm contamination has been proven by numerous studies (Ban and Kang 2016; Jung et al. 2018; KIM et al. 2019; Lim et al. 2019; Hussain et al. 2019; Venkatesh et al. 2009; Francolini and Donelli 2010).

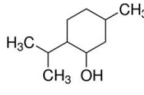
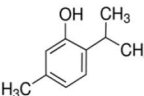
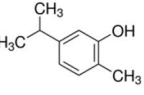
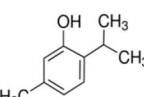
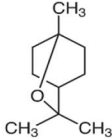
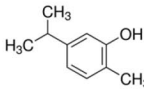

In healthcare and food ecosystems, disinfection must be carried out economically and safely, by reducing the frequency of disinfection, and in the shortest timeframe possible, with the lowest use of chemicals, labor costs, and energy, producing the least amount of waste and without damaging the equipment. Thus, Hurdle technology could be more effective in controlling biofilms compared to the single use of disinfectants. Potential solutions for combined disinfection procedures must therefore be carefully selected to achieve an effective disinfection effect.

Previous studies have shown that the combined use of physical and chemical disinfection strategies is very efficient against biofilms (Vankerckhoven et al. 2011; Kim et al. 2016; Jung et al. 2018). Indeed, the combined treatment of biofilms with ultraviolet irradiation (234 mJ/cm<sup>2</sup>) and hydrogen peroxide (5 ppm) proved to be 10 times more effective than treatment with hydrogen peroxide alone, which could lead to a more eco-friendly treatment (Vankerckhoven et al. 2011). In addition, treatment with biocide solutions that contain more than one bioactive agent was also found to be effective in removing biofilms from industrial surfaces (Ortega Morente et al. 2013). It has been also reported that the combination of different disinfectant compounds can facilitate their diffusion into the biofilm matrix and improve their oxidative activity, resulting in high bactericidal activity even at low concentrations (Ríos-Castillo et al. 2017). Dhowlaghar et al. (2018) demonstrated that the use of a mixture of hydrogen peroxide and quaternary ammonium disinfectants or hydrogen peroxide, octanoic acid, and peracetic acid was able to completely remove *Listeria monocytogenes* biofilm from stainless steel surface, whereas treatment with a single active component in the disinfection procedure could not eliminate biofilm cells completely. In addition, the combined use of EDTA, ethanol, N-acetylcysteine, and recombinant human talactoferrin with amphotericin B, fluconazole, nafcillin, and vancomycin has been successfully applied as catheter lock solutions to rescue colonized catheters. It was found that these combinations were effective in inhibiting both monomicrobial and polymicrobial biofilms of *Staphylococcus epidermidis* and *Candida albicans* (Venkatesh et al. 2009).

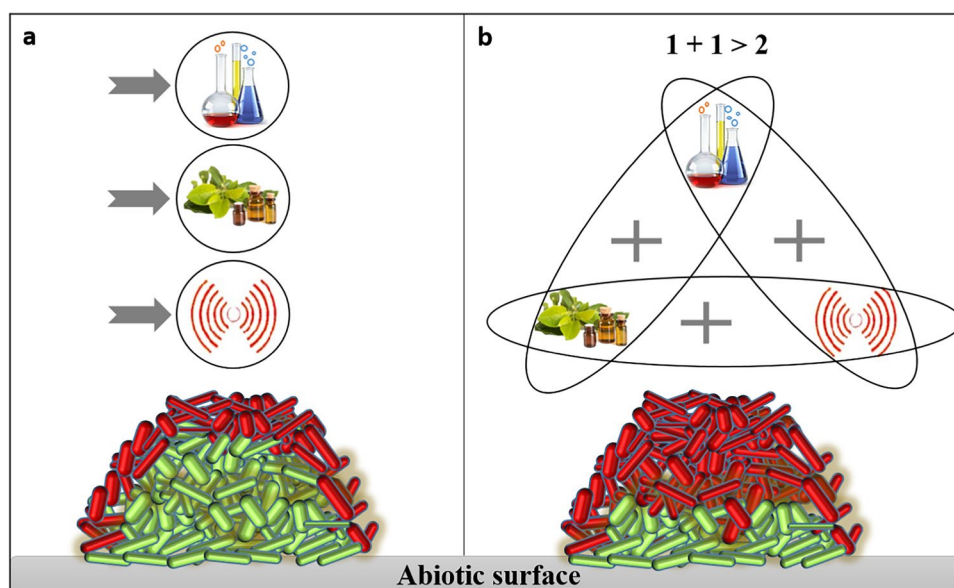
The use of enzymes for the removal of biofilms in the industrial settings generally misses biocidal activity, making them unsuitable for bactericidal applications. To solve this problem, a combined use of enzymatic and antibacterial control approaches is desirable, as the action of the enzyme would contribute positively to the antibacterial activity of the disinfectant (Table 4). This strategy has the potential advantage of avoiding the overuse of toxic antimicrobial agents.

Many studies demonstrate that the antimicrobial agent use after enzymatic treatment can significantly inactivate microbial cells in biofilms (Table 4). EOs are biosourced

**Table 3** Examples of application of essential oil or their components as anti-biofilm, their chemical structure, and targets

Essential oil	Chemical structure	Targets Biofilm	Results	References
<b>Menthol</b>		<i>Klebsiella pneumoniae</i>	75.3-97.5% biofilm inhibition	(Mohamed et al. 2018)
<b>Thymol</b>			85.1-97.8% biofilm eradication	
<b>Peppermint EO</b>			69,2 to 98,2 % biofilm inhibition	
<b>Thyme EO</b>			80,1 to 98,0 % biofilm eradication	
<b>Carvacrol</b>		<i>Staphylococcus aureus</i>	1.87-2.04 log CFU/cm <sup>2</sup> biofilm eradication	(Engel et al. 2017)
<b>Thymol</b>			1.47-1.76 log CFU/cm <sup>2</sup> biofilm eradication	
<b>R. officinalis EO</b>		<i>Staphylococcus epidermidis</i>	>57% biofilm inhibition 67% biofilm eradication	(Jardak et al. 2017)
<b>E. globulus EO</b>		<i>Staphylococcus aureus</i>	74.74 to 89.15 % biofilm eradication Effective biofilm inhibition	(Merghni et al. 2018)
<b>1,8-cineole</b>			77.46 to 90.81 % biofilm eradication Effective biofilm inhibition	
<b>M. longifolia EO</b>		<i>Enterococcus faecalis</i> , <i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Klebsiella pneumonia</i> <i>Candida albicans</i>	Effective anti-biofilm activity	(Pazarci et al. 2019)
<b>Carvacrol</b>		<i>Salmonella typhimurium</i>	5.12 log CFU/cm <sup>2</sup> biofilm eradication	(Trevisan et al. 2018)
<b>Carvacrol</b>		<i>Staphylococcus aureus</i>	6 log CFU/cm <sup>2</sup> biofilm eradication 3 log CFU/cm <sup>2</sup> biofilm inhibition	(Nostro et al. 2009)
			<i>Staphylococcus epidermidis</i>	

**Fig. 4** Improved biofilm cell reduction by hurdle technology: **a** single disinfection strategy using a chemical, physical, or biological method; **b** combined disinfection strategies using chemical-chemical, physical-chemical, or biological-chemical methods



compounds used as alternative natural disinfectants suitable for biofilm control. In addition, it has been reported an increased activity of these natural antimicrobials to inactivate biofilms when combined with other methods (Table 4). Thus, the use of enzymes in combination with an EO as biological hurdles seems to be a promising strategy to control biofilms. The goal of this strategy is that enzymes disrupt and destroy the biofilm matrix, so that the biosourced antimicrobials can hit the target efficiently and easily.

### Encapsulation as a tool to improve antibiofilm compound activities

Microencapsulation aims in protecting the bioactivity of solid, liquid, or gaseous materials by trapping within a surrounding matrix forming particles with a diameter of 1 to 1000  $\mu\text{m}$  (Fu and Hu 2017). Microparticles can be in the form of microspheres or microcapsules. Microspheres are matrix systems in which the core is dispersed (heterogeneous microsphere) and/or dissolved in a polymer matrix (homogenous microsphere) (Silva et al. 2003), while microcapsules are particles consisting of an inner core surrounded by a material that is significantly different from that of the core. Mononuclear and polynuclear microcapsules can be classified according to the division of the core or not. However, the terms microcapsules and microspheres can be used synonymously (Singh et al. 2010).

### Coating materials for microencapsulation

The coating serves as a protective film for isolating the core from inadequate exposure; the core can be released in the ideal place or at the ideal time, in various manners

depending on the characteristics of the coatings material, such as physical pressure, friction, diffusion, dissolution of the wall, and biodegradation (Suave et al. 2006; Qin 2016). The appropriate selection of wall material is extremely critical as it has a significant impact on the effectiveness and stability of the microcapsule. The most appropriate wall material should have the same properties: controlled release under specific conditions; nonreactive with the core; capacity to hold and stabilize the core inside the capsule; ability to protect the core from unfavorable conditions; absence of disagreeable taste in case of food application; and economic feasibility (Nazzaro et al. 2012; Gharsallaoui et al. 2012). A variety of coating materials can be used in microencapsulation such as synthetic polymers such as nonbiodegradable polymers (e.g., poly methyl methacrylate (PMMA), acrolein, glycidyl methacrylate epoxy polymers) (Kreuter et al. 1983; Margel and Wiesel 1984) and biodegradable polymers (e.g., lactides, glycolides, and their copolymers) (Wakiyama et al. 1981); and natural polymers such as proteins (e.g., albumin, gelatin, collagen) (Toshio et al. 1981), carbohydrates (e.g., agarose, carrageenan, chitosan, starch) (Patel et al. 2011), and chemically modified carbohydrates (e.g., poly dextran, poly starch) (Jain 2000).

### Control of the release of encapsulated molecules

Encapsulation should protect and isolate the core from the environment until the desired release at the appropriate time and place (Gouin 2004). Many factors affect the rate of releasing including the interactions between wall material and core, the volatility of core, the ratio of core to support material, the size, and viscosity of particle of wall material, among others (Roberts and Taylor 2000).

**Table 4** Hurdle technology using enzymes and essential oils combined with another technology for biofilm control

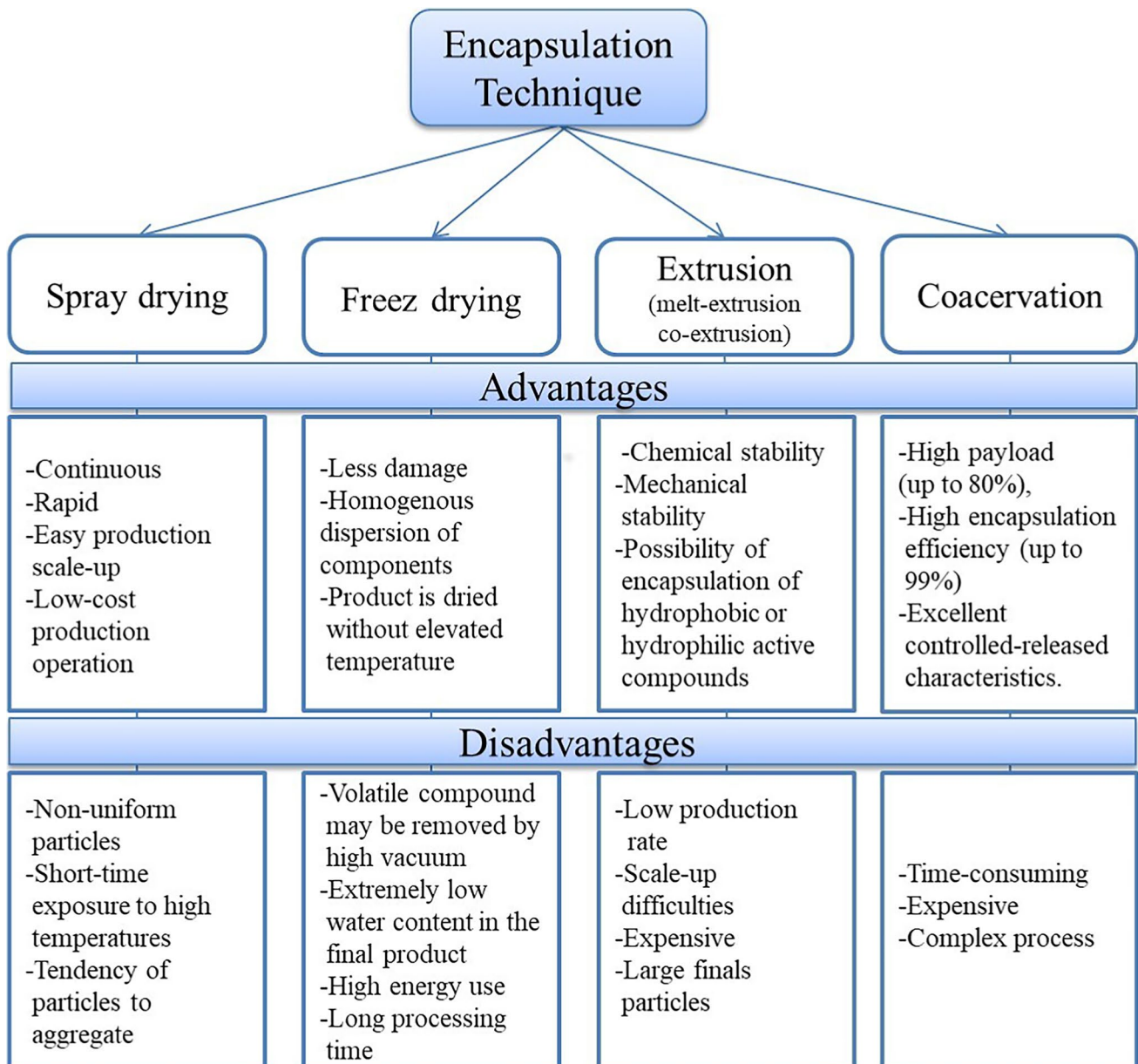
Hurdle technology	Technique	Target biofilm	Finding	References
Enzymes + other technology	Protease K + sodium hypochlorite	<i>Escherichia coli</i> O157:H7	Increased sensitivity of biofilm cells (6.15 log CFU/cm <sup>2</sup> biofilm reduction)	(Lim et al. 2019)
	Pronase + benzalkonium chloride	<i>Listeria monocytogenes</i> - <i>Escherichia coli</i> dual-species biofilm	Reducing dual-species biofilm cells below the detection limit Preventing secondary colonizers from further adhesion after the antimicrobial treatment	(Rodríguez-López et al. 2017)
	Cellulase + cetyltrimethyl ammonium bromide	<i>Salmonella</i>	Completely remove biofilm (6.22 log CFU/cm <sup>2</sup> biofilm reduction)	(Wang et al. 2016)
	Polysaccharide depolymerase enzymes + chlorine dioxide	<i>Klebsiella</i> spp.	Increase of the disinfection effect (92% biofilm removal) Reducing the attachment of bacteria as well as the adhesion of EPS in the biofilm	(Hansen et al. 2019)
	Savinase + shear stress	<i>Pseudomonas aeruginosa</i>	Completely removing of biofilm (90% biofilm removal)	(Pechaud et al. 2012)
	Protease type XXIII, crystalline trypsin, amyloglucosidase, lysozyme, papain + ultrasonic waves	<i>Escherichia coli</i>	61–96% Biofilm removal	(Oulahal-Lagsir et al. 2003)
Essential oils + other technology	<i>Lippia sidoides</i> , <i>Thymus vulgaris</i> , <i>Pimenta pseudocharyophyllus</i> + peracetic acid	<i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i>	Doses required to reach a reduction greater than 4 log CFU/cm <sup>2</sup> in biofilms were reduced	(Vázquez-Sánchez et al. 2015, 2018)
	Clove oil + cold nitrogen plasma	<i>Escherichia coli</i> O157:H7	5.48 log CFU/cm <sup>2</sup> reduction of biofilm was achieved by synergetic treatment	(Cui et al. 2016b)
	Thyme oil, thymol + ciprofloxacin	<i>Klebsiella pneumoniae</i>	Strong synergistic activities which affect the viability of biofilm cells (decrease ciprofloxacin effective dose)	(Mohamed et al. 2018)

The release of the core is conditioned by several mechanisms involved: degradation (enzymes such as lipases and proteases that degrade lipids and proteins, respectively) (Rosen 2005), diffusion (chemical properties of core and wall material and physical properties of wall determine the releasing of core from intact wall) (Choudhury et al. 2021), use of solvent (contact with solvent that dissolve wall material) (Frascareli et al. 2012), pH (solubility of membrane wall altered by changes of pH) (Toldrá and Reig 2011), pressure (applied pressure to the capsule wall cause releasing) (Wong et al. 2009), and temperature (expanding or collapsing of wall material in a critical

temperature which name temperature-sensitive release or melting of wall material when the temperature increase which name fusion-activated release) (Park and Maga 2006). Moreover, the combination of two or more mechanisms can be used (Desai and Park 2005).

**Microencapsulation methods**

Many encapsulation methods such as spray drying, extrusion, and coacervation are currently used for the encapsulation of the antimicrobial substances (Fig. 5). The choice of the most appropriate method depends on the



**Fig. 5** Examples of different techniques for antimicrobials encapsulation with some advantages and disadvantages (Dolça et al. 2015; Bakry et al. 2016; Wang et al. 2018; Khairnar et al. 2012; Dajic Stevanovic et al. 2020)

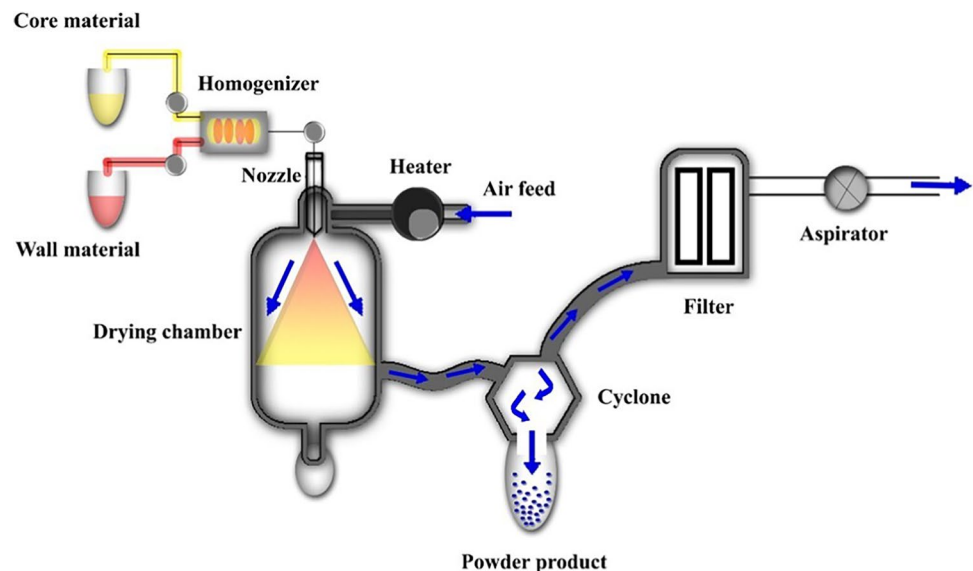
capsule application, the type of core, the chemical and physical characteristics of the core and wall, the particle size required, the mechanism of release required, the scale of production, and the cost (Suave et al. 2006). The spray drying technique is the most common encapsulation method that has been used for decades to encapsulate mainly flavors, lipids, and pigments (Gharsallaoui et al., 2007). Several applications of spray drying in industrial field range from encapsulation of fragrances and flavors in food industries to pigments in manufacture (Laohasongkram et al. 2011). In addition, this technique is widely used for the encapsulation of enzymes and antimicrobials substances such as EOs to ensure their activities (Schutyser et al. 2012; Dajic Stevanovic et al. 2020). However, the optimal choice of drying conditions and adapted matrix formulations is necessary to avoid serious thermal damage leading to a loss of enzymatic activity or volatility of the essential oil (Gharsallaoui et al. 2007; Schutyser et al. 2012). Spray drying is a relatively inexpensive and commercially feasible method of microencapsulation. Biomolecules used as carriers for this technique are starch, maltodextrins, chitosan, and gum Arabic (Dajic Stevanovic et al. 2020). Spray drying microencapsulation involves 4 steps as shown in Fig. 6: (1) preparation of the emulsion, (2) homogenization of the emulsion, (3) atomization of the dispersion, and (4) dehydration of the atomized particles (Bakry et al. 2016). This process consists of forming an emulsion, suspension, or solution containing the wall material and core; then pulverization in a drying chamber in which circulates hot air, upon contact with the hot air, the water evaporates immediately, and the core is encapsulated into the wall material (Laohasongkram et al. 2011).

### Role of microencapsulation in biofilm control

Microencapsulation is widely used in the fields of medicine, food, cosmetics, pharmaceuticals, textiles, agriculture, and advanced materials, which make this technique widely used in the encapsulation of active constituents: enzymes, EOs, flavors, colors, sweeteners, microorganisms, etc. (Desai and Park 2005; Fu and Hu 2017). One of the recent use of microencapsulation is the control of biofilm on industrial equipment and materials and by the encapsulation of antimicrobial substances to ensure their stability and long-term activity, as well as to limit their interactions with the biofilm matrix components (Khelissa et al. 2021a, b).

As mentioned earlier, the use of enzymes is an important tool to remove biofilms through the enzyme ability to degrade the EPS and destroy biofilms (Xavier et al. 2005). Thus, extensive researches have been conducted to immobilize enzymes in mechanically resistant capsules, in most cases in dry microcapsules, in order to protect enzymes during storage and to control their release (Mohamad et al. 2015). Microencapsulation is a promising strategy to prevent and stabilize enzymes under severe reaction conditions from denaturation by proteolysis and dilution effects, and thus maintain high catalytic activity (Chaize et al. 2004; Tetter and Hilvert 2017; Zdarta et al. 2018). Orgaz et al. (2007) demonstrated that the combination of delayed-release encapsulated pronase with cellulase, pectin lyase, or esterase leads to three to four decimal reduction in cells and detach up to 90% of the biofilm of *Pseudomonas fluorescens* after 2 h at 25 °C. These data prove that these results are more favorable than those obtained with the same application of the equivalent soluble enzyme mixtures. Tan et al. (2020) show that the co-immobilization of deoxyribonuclease I (DNase) and cellobiose dehydrogenase (CDH) results in a bifunctional

**Fig. 6** Schematic representation of the spray drying microencapsulation process





particle that targets both the microorganisms and the biofilm matrix. The assessment of the antibiofilm activities of these particles has shown a high ability to penetrate through the biofilm matrix and interfere with microbial cells, thus exhibiting a stronger activity to inhibit biofilm formation as well as to disrupt preformed biofilms.

Furthermore, the use of EOs to control biofilm has been extensively studied in recent years; a wide variety of EOs can be used as antibiofilm compounds. However, EOs are not stable and can be degraded in the presence of oxygen, light, and temperature. Thus, efforts have been attempted to protect them by encapsulation in various colloidal systems such as microspheres, microcapsules, liposomes, and nanoemulsions (Sherry et al. 2013). Many studies show that protecting the EOs with antimicrobial activity in a capsule can increase their bioactivity and efficiency to remove biofilm from surfaces (Dohare et al. 2014; Duncan et al. 2015; Cui et al. 2016a), as well as decreasing volatility and improving stability and water solubility (Bilia et al. 2014). For example, peppermint oil encapsulated in starch-based emulsions showed increased stability and bioavailability characteristics and improved activity against *Staphylococcus aureus* and *Listeria monocytogenes* relative to free EOs (Liang et al. 2012). The antibacterial activity of EOs after nanoencapsulation has been shown to very often exceed the efficacy of the current antibiotic (Zaman et al. 2017). Dohare et al. (2014) reported that the encapsulation of Eucalyptus globulus oil has increased its antibiofilm activity against *Escherichia coli* biofilm from 62 to 81% compared to the soluble one; thus, the use *Eucalyptus globulus* oil encapsulated into a nanoparticle is important for controlling biofilm associated with microbial infections and diseases. Other studies showed that the two antimicrobials, carvacrol and eugenol, encapsulated in micellar nonionic surfactant solutions, were significantly effective against two strains of *E. coli* O157:H7, reducing viable counts by 3.5 to 4.8 log CFU/cm<sup>2</sup> within 20 min of exposure (Pérez-Conesa et al. 2011). Furthermore, Cui et al. (2016a, b) evaluated the anti-biofilm effect of cinnamon oil, encapsulated in liposomes, on methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms. The results showed that the use of liposomes improves the stability of cinnamon oil, which has an effective antibacterial performance on MRSA and its biofilms and prolongs the time of action.

## Conclusion

The prevention/eradication of biofilms in the industrial and medical sectors is one of their main concerns. This field can provide an appropriate environment for the development of biofilms that threaten public health and increase economic losses. A clear understanding of the mechanisms of biofilm formation and resistance to disinfecting agents

is necessary to provide an effective strategy to prevent and destroy biofilms. Biofilm resistance to disinfectants appears to be multifactorial and involves several parameters. In addition, the side effects caused by these agents require the search for alternative natural antimicrobial agents to obtain the requested treatment and overcome the disadvantage of the conventional antimicrobial used. The biofilm matrix is the main physical barrier preventing the penetration of biocides into biofilms. Therefore, if one or more compounds capable of destroying the structural components of the matrix produced by biofilm as well as active against the microbial biofilm are found, then the “microbe city” (biofilm) would be permanently destroyed and eradicated. However, the activity and stability of the anti-biofilm agents used may be affected by several parameters. On this basis, the encapsulation of these compounds can be useful to protect and ensure their stability and activities against matrix biofilm as well as biofilm-producing microorganisms, in order to prevent the formation and/or eradicate the establishing biofilms.

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## Declarations

**Ethics approval** Not applicable, since the work does not involve any study with human participants or animals.

**Consent to participate** Not applicable.

**Consent for publication** All co-authors have given their consent to publish this manuscript.

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