The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 19(2): S.I (1), 400-408, 2024 www.thebioscan.com

Biofilms in Food Processing: A Comprehensive Review of Understanding Formation, Challenges, and Future Directions

***Ligi Lambert D Rosario[1], Harsha Mohan[2], Febiya Anna Fedin[3], Sona S Dev[4]**

[1&4]PG and Research Department of Biotechnology, St. Peter's College, Kolenchery, Ernakulam, 682311, Kerala, India

[2&3] School of Biosciences , Mahatma Gandhi University, P. D .Hills, Kottayam 686560.

*e-mail: ligi.lambert@stpeterscollege.ac.in

*Corresponding Author: **Ligi Lambert D Rosario**

DOI: https://doi.org/10.63001/tbs.2024.v19.i02.S.I(1).pp400-408

KEYWORDS
Biofilm,
Extracellular polymeric substances (EPS),
food processing,
bacteria

Received on:

08-08-2024

Accepted on:

28-11-2024

ABSTRACT

Biofilms are complex communities of microorganisms attached to the surfaces and can cause cross-contamination of foods. Biofilms are irreversibly linked with a surface inside an extracellular polymeric substance matrix (EPS) which is challenging for the food industry. There exists an urgent need for disinfectants or new technologies to restrict reversible and irreversible attachment which are the main cause of surface tension of microorganisms that leads to surface adhesion. Many articles have reported the negative effects of biofilm production in the food industry and the role of microorganisms such as *Bacillus cereus*, and Listeria monocytogenes in foodborne diseases. This review paper discusses the formation and characteristics of Biofilm, the structure and composition of biofilms, factors influencing biofilm development, the presence of biofilms in food processing environments, the type of microorganisms present in the biofilms, and strategies and controlling measures for preventing biofilm formation. The article also discussed some positive impacts of biofilms in various applications.

INTRODUCTION

Biofilms belong to a group of microorganisms including bacteria which can live and reproduce as a unit known as a colony (Bjarnsholt et al. 2013a). These biofilms are particularly living biomass possessing a sophisticated complex structure that continues to challenge researchers in this field. Their structure protects and enables the growth of their colonies. Modern understanding of biofilms defines them as an immobile and complex structure comprised of single or multiple species of bacteria, cellular by-products, and host cells, in which the cells are attached irreversibly to the surface and encased in an extracellular polymeric substance produced by bacteria (Bjarnsholt et al. 2013b).

The complex three-dimensional architecture of Biofilm formation is a multi-step process. These stages involve the initial reversible attachment of planktonic microorganisms to a pre-conditioned surface, followed by a transition to irreversible attachment as the biofilm forms, facilitated by the production of extracellular polymeric substances (EPS). Microcolonies then develop into mature biofilm, and cells eventually disperse from the biofilm into the surrounding environment. The key feature involves the dynamic simulation between the microorganisms and their environment. Biofilms pose significant challenges in food industries due to their physical structure, which makes them more defensive to environmental stressors such as antimicrobials and disinfectants (Olanbiwoninu and Popoola 2023).

In food processing units, biofilms can cause severe contamination and foodborne illness due to their association with pathogenic bacteria. The surfaces used in food processing including glass, polyethylene, and stainless steel are the underlayers for biofilm formation. Listeria, Campylobacter, and Salmonella are the common pathogens capable of forming biofilms on these surfaces with organic residues which will promote their growth and can cause significant health risks. Understanding the environmental factors such as nutrient availability and fluid dynamics, which can promote the growth of biofilms are the key factors for developing a targeted intervention (Sharma et al. 2023). Despite these challenges, biofilms are universally harmless. In certain cases, biofilms play beneficial roles such as in fermentation processes, where the microbial communities associated with it can enhance the biochemical and sensory properties of food products. Even so, the key focus will be on mitigating the risk associated with forming pathogenic biofilms to assure food safety.

BIOFILM FORMATION AND CHARACTERISTICS:

Mechanisms of biofilm formation

Biofilm development is a complex process that occurs in five distinct stages: initial attachment, bacterial aggregation, microcolony formation, maturation, and dispersion. The process begins with the initial attachment of free-floating planktonic bacteria to surfaces, mediated by bacterial appendages such as pili and flagella, as well as other physical forces (Gupta et al. 2015; Joo and Otto 2012; Speziale and Geoghegan 2015). This attachment is often transient and reversible, influenced by factors including temperature, pressure, bacterial properties, and surface composition (Büttner, Mack, and Rohde 2015).

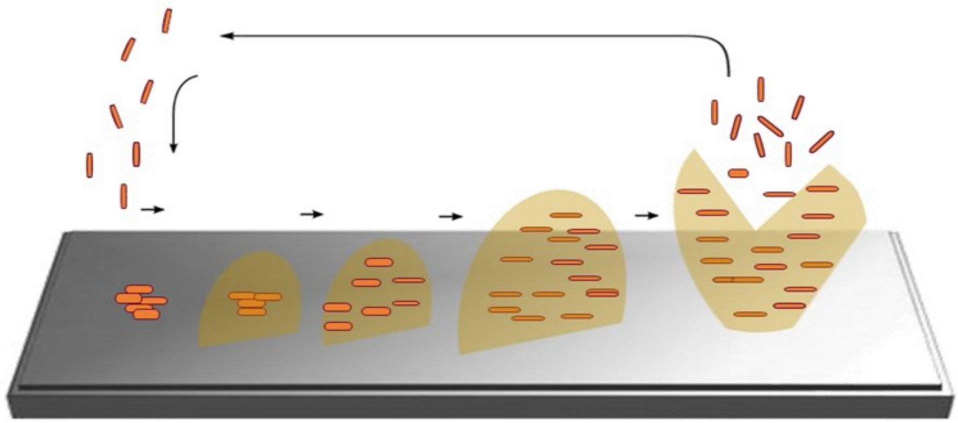

Figure 1: Stages of biofilm formation

In the bacterial adhesion and aggregation phase, bacteria undergo a more stable adhesion process referred to as the anchoring or latching phase (An, Dickinson, and Doyle 2000). This involves molecularly coordinated binding between specific adhesins on the bacterial surface and the substratum, facilitated by the production of extracellular polymeric substances (EPS) that interact with surface materials and receptors (Leung et al. 1998; Vacheethasanee and Marchant 2000). This results in a permanent attachment of bacteria to the surface. Following adhesion, microcolony formation occurs where bacterial cells multiply and form microcolonies within the EPS matrix. This stage is driven by chemical signaling and the formation of micro-communities, which are essential for waste elimination, nutrient flow, and substrate exchange (Costerton, Stewart, and Greenberg 1999; McKenney et al. 1998).

In the maturation phase, biofilm structure becomes more complex as bacterial cells secrete signaling factors that enhance biofilm stability and protect against antimicrobial agents. The EPS matrix strengthens and protects the biofilm, facilitating further bacterial growth and biofilm maturation(Gupta et al. 2015). The final stage, dispersion, involves the release of bacteria from the biofilm into the surrounding environment, enabling their spread and potential to cause infections. This process is crucial for biofilm expansion and can lead to chronic infections and severe conditions like embolic complications, necessitating prompt medical intervention (Veerachamy et al. 2014).

Structure and Composition of Biofilms

Biofilms are intricate microbial communities embedded in a self-produced extracellular matrix (ECM) composed of extracellular polymeric substances (EPS), including polysaccharides, extracellular DNA (eDNA), and proteins. This matrix accounts for 75% to 90% of the biofilm's structure, providing a sticky, resilient framework that binds cells together and facilitates nutrient flow (Boels 2011; Lu and Collins 2007). The remaining 10% to 25% comprises microbial cells. The ECM also contains water channels or interstitial spaces that aid in nutrient cycling and waste removal.

Advanced imaging techniques, such as confocal scanning laser microscopy, have enhanced our understanding of biofilm structure, revealing its complex arrangement of microbial cells and EPS. The biofilm structure and composition vary based on environmental conditions, microbial species, and nutrient availability. In addition, biofilms in different environments may include noncellular elements like corrosion particles, blood components, and mineral crystals. Biofilms in water systems are more diverse and complex, whereas those in medical devices often consist of single-species communities with a mix of diatoms, bacteria, and environmental debris.

Factors Influencing Biofilm Development

Biofilm formation is influenced by various factors, including the chemical composition of the substratum, pH, temperature, water current and oxygen concentration. Substratum materials, such as rubber, glass, stainless steel, and polymers, can support biofilm growth (Chia et al. 2009). Temperature variations in different environments, from 18°C in freezers to over 100°C in sterilizers, affect biofilm characteristics by influencing the physiological state of bacterial cells and the physical properties of compounds (Villain-Simonnet, Milas, and Rinaudo 2000). Oxygen concentration also plays a crucial role, as microorganisms adapt to varying levels of oxygen diffusion in their environments, whether attached to surfaces or free-living in aquatic settings (Morris and Schmidt 2013). The bacterial cell type factors like strains/species, production of EPS , gene expression and Quorum sensing systems in bacteria influences the production of biofilm.(Fig:2). QS plays a crucial role in biofilm maturation processes because bacteria regulate collective behavior and track cell density (Zhao.et al 2017).

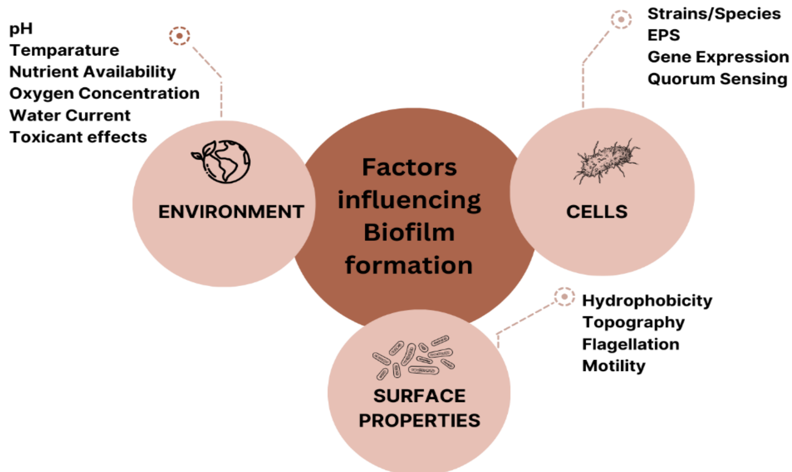

Figure 2: Biofilm architecture and function are the product of a complex interactions (Whitehead & Verran 2015).

BIOFILMS IN FOOD PROCESSING ENVIRONMENTS:

The formation of microbial biofilm is a very complex process. Firstly, organic molecules from food are deposited on the surfaces of equipment. Secondly, biologically active microorganisms are attracted to the conditioned surfaces. Thirdly, some microbial cells remain even after cleaning and sanitizing and initiate growth. Lastly, larger biofilms are formed with the help of expression of genes and quorum sensing. In the process of biofilm formation, properties of substratum and cell surfaces, surrounding environmental factors, and genetic regulation of bacteria play an important role in reversible or irreversible attachment, and micro-colony formation to a large biofilm(Langsrud 2015).

The growth of biofilms is highly influenced by the solid surface facilities in the food processing industry. The physical characteristics of the solid surfaces such as critical surface tension facilitate the initial attachment of microbial communities. Bacterial adhesion is highly promoted in high-energy and wet surfaces. Hydrophilic regions such as stainless steel, glass, etc. are the most common sites for bacterial cell attachment than hydrophobic regions like Buna-N rubber and other plastics. Stainless steel type 304, is an ideal material widely used in the fabrication of equipment in the food-processing industry due to its physico-chemical stability and high corrosion resistance. Similarly, Teflon and other plastics have been used in the production of gaskets and accessories of instruments. With continuous reuse, these types of surfaces will become rougher and it protects bacteria from shear forces in the food fluid.

The bacterial attachment also depends on the conditioning of the substratum. The organic molecules such as proteins from pork, beef, milk, and even the EPS produced by bacteria could form a film around the substratum. It is found that many of the food-contact surfaces such as Teflon and Stainless steel have been found to attract the milk proteins and form conditioned substrata, which may inhibit or encourage the bacterial attachment based on the concentration of milk. The substratum conditioned by diluted milk facilitates bacterial attachment more easily than that of whole milk and it was also assumed that the presence of some proteins like bovine serum album (BSA) inhibits the attachment of bacteria to various surfaces. In summary, the initiation of bacterial attachment is influenced by the surface properties of the conditioned substrate.

Most Relevant Biofilms in the Food Industry

In the food industry, biofilms are a significant concern due to their ability to form on various surfaces, leading to contamination and spoilage. The diverse substrates found in food processing environments, such as wood, glass, stainless steel, polyethylene, rubber, and polypropylene, can act as surfaces for pathogenic biofilm formation. This poses challenges in cleaning and disinfection, making it crucial to understand and manage these biofilms effectively. Below are some notable examples of biofilm-forming pathogens relevant to the food industry:

1.Listeria monocytogenes

Listeria monocytogenes is a Gram-positive bacterium known for its ability to form biofilms on surfaces like polypropylene, steel, rubber, and glass. This pathogen is a major concern in the food industry due to its persistence and potential to cause serious health issues such as septicemia, meningitis, and in pregnant women, miscarriage or stillbirth. It is listed among the top five foodborne pathogens by the World Health Organization (WHO) (Langsrud 2015). The bacterium can thrive in various environments, including soil, water, and animal feces, and poses a significant threat due to its resistance to common disinfection methods (Rothrock et al. 2017).

2.Pseudomonas spp.

Pseudomonas spp. are Gram-negative bacteria that are well-studied for their biofilm-forming capabilities. They are commonly found in refrigerated foods, particularly those high in protein such as meat, poultry, and dairy products. These bacteria are known for their resistance to conventional cleaning methods and can cause food spoilage, characterized by rancidity, off-odors, and pigmentation changes (Korber, Mangalappalli-Illathu, and Vidovic' 2009). Pseudomonas biofilms are particularly problematic due to their ability to persist in both solid-liquid interfaces and complex food processing environments.

3.Shewanella putrefaciens

Shewanella putrefaciens is frequently associated with food spoilage, especially in marine environments. It thrives under various temperature conditions and can produce volatile sulfur compounds responsible for off-flavors in meat, poultry, and seafood. Its biofilm formation presents significant challenges for food processing industries, impacting the quality and safety of products (Carrascosa et al. 2021; Korber, Mangalappalli-Illathu, and Vidovic' 2009).

4.Salmonella enterica

Salmonella enterica, a Gram-negative bacterium, is notorious for causing foodborne illnesses such as gastroenteritis and septicemia. It can form complex biofilms on surfaces like stainless steel, exhibiting a range of morphologies depending on nutrient availability. Salmonella biofilms are a major concern in food processing plants due to their potential for cross-contamination and the risk of outbreaks (Wang et al. 2016).

5.Bacillus cereus

Bacillus cereus is a Gram-positive, spore-forming bacterium that exhibits remarkable resilience to heat, chemicals, and radiation, allowing it to persist in various environments from 4°C to 50°C (Bottone 2010). This bacterium is commonly found in soil and can contaminate a range of food products, including dairy, rice, vegetables, and meat. Its spores enable it to endure pasteurization, making it a frequent contaminant in dairy products and other foods (Carrascosa et al. 2021). *B.*

cereus forms biofilms on food contact surfaces like stainless steel pipes, conveyor belts, and storage tanks. These biofilms can produce toxins leading to severe foodborne illnesses with emetic and diarrheal symptoms (Galié et al. 2018a). Additionally, B. cereus biofilms secrete various bacteriocins, metabolites, surfactants, and enzymes such as proteases and lipases, which can negatively affect food quality (Grigore-Gurgu et al. 2019). Although its flagella are not directly involved in adhesion to surfaces like glass, they play a crucial role in motility and spreading on non-colonized surfaces, contributing to effective biofilm formation (Carrascosa et al. 2021; Houry et al. 2010). The persistence of *B. cereus* in food processing environments presents significant challenges for contamination control.

6.Enterohemorrhagic Escherichia coli (EHEC)

Enterohaemorrhagic Escherichia coli (EHEC), particularly the O157 serotype, is a significant foodborne pathogen known for causing severe diseases such as bloody diarrhea and hemolytic uremic syndrome (HUS) (Carter et al. 2016; Gould et al. 2013). This bacterium is characterized by its ability to form biofilms on various surfaces within food processing environments, which complicates control measures. EHEC utilizes pili, flagella, and membrane proteins for attachment and biofilm formation, enhancing its resistance to disinfectants and contributing to its persistence (Carrascosa et al. 2021; Galié et al. 2018b). The widespread dissemination of E. coli in natural environments is largely attributed to its biofilm-forming capabilities, making it a serious concern for food safety. Despite the challenges posed by EHEC biofilms, there is no effective means to prevent their formation or treat EHEC infections comprehensively, as antibiotic treatment can exacerbate conditions like HUS and kidney failure (Lee et al. 2007).

7.Campylobacter jejuni

Campylobacter jejuni is a Gram-negative bacterium that forms biofilms under both microaerophilic and aerobic conditions. It is a common cause of bacterial gastroenteritis and can survive outside the avian intestinal tract, contaminating food products like unpasteurized milk (Chlebicz and Śliżewska 2018). Biofilm formation by C. jejuni in food processing environments can contribute to its persistence and pathogenicity (Carrascosa et al. 2021; Klančnik et al. 2020).

8.Geobacillus stearothermophilus

Geobacillus stearothermophilus is a thermophilic, spore-forming bacterium that can form biofilms on stainless steel surfaces in processing equipment such as evaporators and heat exchangers. Its ability to produce spores and survive in high temperatures makes it a challenge in the dairy industry, where it can contaminate milk during processing (Carrascosa et al. 2021; Wu et al. 2019).

9.Anoxybacillus flavithermus

Anoxybacillus flavithermus is a Gram-positive, thermophilic bacterium that forms biofilms in the dairy industry, particularly in milk powder processing. Its spores are highly heat-resistant, and its vegetative cells can grow at high temperatures, leading to contamination issues in milk powder production (Carrascosa et al. 2021; Sadiq et al. 2017).

10.Pectinatus spp.

Pectinatus spp. are anaerobic Gram-negative bacteria found in breweries, particularly in unpasteurized beer. These bacteria form biofilms due to sanitation problems and can cause spoilage in the brewing industry, affecting the quality of the beer (Carrascosa et al. 2021; Paradh, Mitchell, and Hill 2011).

11.Synergistic Pathogens

In food processing environments, mixed-species biofilms can form, where pathogens such as *Aeromonas hydrophila, Listeria monocytogenes, Salmonella enterica, and Vibrio spp.* Interact synergistically. These interactions can complicate control measures and contribute to significant health and economic issues. Synergistic interactions in biofilms can be influenced by quorum sensing, which regulates biofilm production and dispersion (Carrascosa et al. 2021; Mizan, Jahid, and Ha 2015). The other pathogens in food spoilage include yeast and fungal biofilms. These are highly adaptable to environmental conditions including extreme temperature and low -pH. Yeast is capable of metabolizing the acid added to foods as preservatives leading to the spoilage of products like fruit juices and dairy items. Certain molds can grow even in the presence of commonly used antimycotics, further complicating food preservation efforts(Huis In't Veld 1996). Overall, controlling biofilm formation by various microorganisms is crucial in maintaining food safety and preventing outbreaks of foodborne illnesses.

DETECTION AND MONITORING OF BIOFILMS

Microbial biofilms are major concerns in the sectors like healthcare, and food industries due to resistance possessed by the bacteria to the available conventional antibiotics and cleaning procedures which will lead to persistent contamination (Table 1).

Table 1: Different strategies for the detection and monitoring of biofilms.

Devices	Systems used	Techniques	Reference
Sensing Devices	Optical sensors	Fiber optics Brillouin spectroscopy Surface Plasmon Resonance (SPR) White light interferometry Localized Surface Plasmon resonance (LSPR)	(Abadian et al. 2014; Funari et al. 2018; Funari and Shen 2022; Mattana et al. 2017)
	Mechanical systems	Quartz crystal microbalance (QCM) Quartz tuning fork oscillators Surface acoustic wave (SAW) Interfacial rheometer and tensiometer	(Berkenpas, Millard, and Pereira da Cunha 2006; Sprung et al. 2009; Waszczuk et al. 2012)
	Electrochemi cal sensors	Impedimetric sensors Potentiometric/amperometric sensors	(Funari and Shen 2022)
Sensor-free devices	Microfluidics	Microfluidic chip for images	(Parvinzadeh Gashti et al. 2016; Pousti et al. 2018)
	Flow cells	Calgary device Robbin's device CDC biofilm reactor	(Ceri et al. 1999; Goeres et al. 2005; Kharazmi, Giwercman, and Høiby 1999)
Biofilm dynamics	Optical systems	Planar optodes Doped micro/nanoparticles	(Funari et al. 2018)
	Electrochemical sensors	Microelectrode probes	(Funari et al. 2018)
	Mechanical systems	Interfacial rheology Tensiometry	(Funari et al. 2018)

***Table 1:** Various innovative methods available for the detection and monitoring of biofilms - Devices, Systems used, and Techniques*

CHALLENGES POSED BY BIOFILMS

Biofilms pose significant challenges in food processing due to their role as reservoirs for potentially harmful microorganisms that can cross-contaminate food and lead to spoilage or foodborne illnesses [52,53]. These microbial communities adhere to surfaces in food processing environments and are notoriously difficult to eradicate with conventional cleaning and disinfection methods. Standard biocides and sanitizers, which are effective against planktonic cells, often fall short in eliminating biofilms due to the protective extracellular matrix and complex structure of these microbial clusters (Chylkova et al. 2017; Corcoran et al. 2014). Research has shown that biofilms exhibit a higher tolerance to sanitizers compared to single-species biofilms, and the effectiveness of biocides is further compromised when microbial communities are exposed to suboptimal concentrations due to improper use or dilution (Alvarez-Ordóñez et al. 2019; Fagerlund et al. 2017; Giaouris et al. 2013).

To combat these challenges, innovative approaches are being explored, including the modification of food processing surfaces to prevent microbial adhesion. Advances in surface coatings, such as diamond-like carbon and fluoropolymer coatings, have demonstrated reduced biofilm formation and improved cleaning efficacy (Gomes et al. 2018; K. Huang, McLandsborough, and Goddard 2016). Additionally, the development of novel disinfectants and antimicrobial agents, including enzymatic detergents and electrolyzed water, offers promising alternatives for biofilm control. Enzymes such as DNase and proteases target the biofilm matrix directly, while electrolyzed water has been effective against various foodborne pathogens (Alvarez-Ordóñez et al. 2019; Brown et al. 2015; Han et al. 2017).

Overall, tackling the biofilm problem requires a multifaceted approach, integrating novel materials, improved disinfection methods, and targeted antimicrobial treatments to enhance food safety and processing efficiency. This comprehensive strategy is crucial for overcoming the limitations of traditional biocides and addressing the persistent issues posed by biofilms in food processing environments (Alvarez-Ordóñez et al. 2019; Kim et al. 2017; Puligundla and Mok 2017).

STRATEGIES FOR PREVENTING AND CONTROLLING BIOFILMS

Several chemical sanitizers can be used for biofilm treatment to decrease the level of microbial population to a level that is safe for humans and the process is called sanitization (Schmidt 1997). It is essential to sanitize the food processing equipment to prevent the cross-contamination of food batches. Chlorine-based sanitizers are commonly been used in the food industries but it is found that some microbes such as *S.enterica* show resistance to chlorine due to its cellulose production prototype (Y. Yang et al. 2016). Similarly, Aqueous ClO2 has also been used in the food industry and found to be effective against the endospores present inside biofilms on steel surfaces (Nam et al. 2014). NaOCl is an effective chemical used for removing biofilms formed on polypropylene and stainless-steel surfaces. However, pathogens like *Cronobacter sakazakii* were resistant to treatment using NaOCl (Bayoumi et al. 2012). H2O2 is another potent oxidizing agent used as a disinfectant in the food industry. It can destroy the biofilm structure even at a concentration of 0.008-5% without any toxic side effects. H2O2 in combination with acetic acid generates a strong oxidant known as peracetic acid which is found to be effective for the treatment of *S. aureus* and *L. monocytogenes* populations (Srey, Jahid, and Ha 2013).

Another toxic gas that show high oxidizing activity is Ozone. It is capable of destroying various microorganisms including viruses, protozoans, and even biofilms by the breakdown of cellular envelopes. It is mainly used in the dairy industry to restrict the growth of mold on cheeses, powdered formulas, and stainless-steel structures (Varga and Szigeti 2016). Quaternary ammonium compounds known as Metaquats were also used in the food industry for the removal of biofilms as they can disrupt the bacterial cell membrane which leads to bacterial lysis. Even though certain strains like L. monocytogenes were found to be resistant against quaternary ammonium sanitizers (Varga and Szigeti 2016). Other less common sanitizing agents including salicylate-based polyanhydride esters were found to inhibit the biofilm formation of S. enterica even in the initial stages (Varga and Szigeti 2016). Similarly, synthetic brominated furanone F202 is also an uncommon sanitizer used to prevent the biofilm growth of bacteria including *S. enterica* and *E. coli* O103:H2 by targeting the flagellar motion of both bacteria (Vestby et al. 2014).

Enzymatic Disruption

As the enzymes have low toxicity and are biodegradable, they are considered to be the most effective countermeasures against biofilm formation and therefore they are extensively used in the food industry as detergents (H. Huang et al. 2014; Torres et al. 2011). As biofilms are mostly composed of organic molecules like proteins and polysaccharides, enzymes like proteases and glycosidases can be employed as the first option for the removal of biofilms (Boels 2011; Meireles et al. 2016). Likewise, Pectin methylesterase is another enzyme used in the bioreactors against biofilm (Torres et al. 2011). Other enzymes like lyases, cellulases, amylases, glycosidases, and DNAses, are widely used in food industries to remove the biofilms formed by bacteria like *S. aureus* and *P. aeruginosa* (Coughlan et al. 2016).

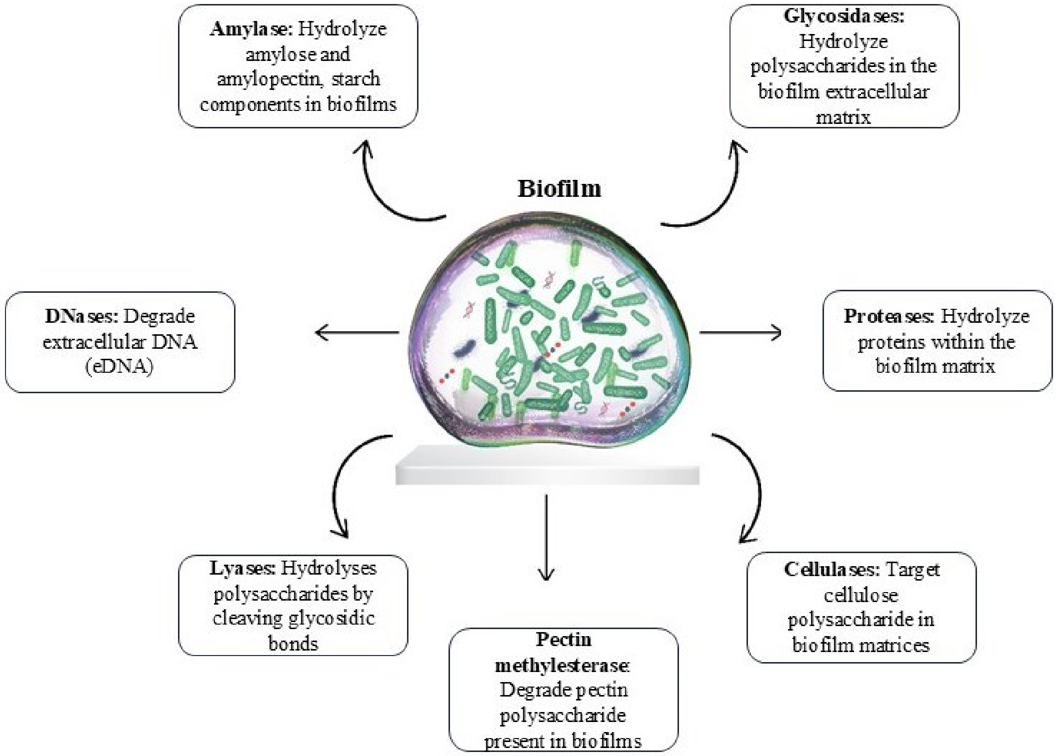

Figure 3: Different types of enzymes used in the food industry for removing biofilms

Bacteriophages

The application of bacteriophages as an anti-biofilm agent is found to be a promising strategy as it can specifically destroy prokaryotic cells (Fister et al. 2016; Iacumin, Manzano, and Comi 2016). The only limitation of this approach is the inability of the bacteriophages to target the bacterial cells inside the biofilm due to the intricate biofilm structure and the existence of extracellular material which will prevent phage diffusion (Pires et al. 2016). However, the phages containing exopolysaccharide depolymerase can enhance the phage invasion and dispersion process through the biofilm under treatment. Endolysins and virion-associated peptidoglycan hydrolases have also been employed as agents for the removal of biofilm as they can penetrate the biofilms more easily (Gutiérrez et al. 2014; Shen et al. 2013).

Other techniques

The resistance of bacteria against the conventional control measures highlights the need for alternative techniques. One of the promising approaches involves nanoparticles due to their unique properties such as large surface-to-volume ratio which distinguishes them from other chemicals (Beyth et al. 2015). Some studies found that the biofilm formation of microbes such as *Geobacillus stearothermophilus* and *Bacillus licheniformis* can be prevented in stainless steel surfaces coated with modified plastic Ni-P-polytetrafluoroethylene (Beyth et al. 2015). Biosurfactants are natural compounds of microbial origin that can bind on the surface of the target microbe and alter its binding ability by reducing surface tension. These can intrude into the microbial cell membranes and alter the permeability, leading to disruption and cell death (Coronel-León et al. 2016). Quorum sensing is a widely distributed intercellular mechanism used by bacteria to regulate gene expression. Inhibition of Quorum Sensing is another important strategy against biofilm formation (Parsek and Greenberg 2005; L. Yang and Givskov 2015).

CONCLUSION

Over the last few years, biofilms have emerged as major concerns in the food industry. It is a relevant topic to be discussed, due to its potential to contaminate foods through biofilm formation and can lead to severe health issues for the public. About 20 % of food poisoning is caused due to biofilms and they exhibit antibiotic resistance up to 1000 times greater than their free-floating (planktonic) counterparts. There are numerous bacterial species which are involved in the biofilm formation. Cleaning and disinfecting the food processing facilities were challenging due to the complex structure of biofilms. For these reasons new technologies need to be developed to eliminate these biofilms, otherwise, they will become a high risk to the public once they form. Many studies have reported the capability of bacteria to form biofilms is greater than the discoveries and elimination of the biofilms is challenging. There exists an urgent need for new non-destructive technologies to understand biofilms and incorporate these findings into the biofilm diagnosis in food industries, for a better understanding of biofilms and microbes and future applications in the food industry.

REFERENCES

- Abadian, Pegah N., Nil Tandogan, John J. Jamieson, and Edgar D. Goluch. 2014. “Using Surface Plasmon Resonance Imaging to Study Bacterial Biofilms.” *Biomicrofluidics* 8(2). /aip/bmf/article/8/2/021804/386091/Using-surface-plasmon-resonance-imaging-to-study.
- Alvarez-Ordóñez, Avelino, Laura M. Coughlan, Romain Briandet, and Paul D. Cotter. 2019. “Biofilms in Food Processing Environments: Challenges and Opportunities.” *Annual Review of Food Science and Technology* 10(Volume 10, 2019): 173–95. https://www.annualreviews.org/content/journals/10.1146/annurev-food-032818-121805.
- An, Yuehuei H., Richard B. Dickinson, and Ronald J. Doyle. 2000. “Mechanisms of Bacterial Adhesion and Pathogenesis of Implant and Tissue Infections” eds. Yuehuei H An and Richard J Friedman. *Handbook of Bacterial Adhesion: Principles, Methods, and Applications*: 1–27. https://link.springer.com/chapter/10.1007/978-1-59259-224-1_1.
- Anand, Sanjeev, and Diwakar Singh. 2013. “Resistance of the Constitutive Microflora of Biofilms Formed on Whey Reverse-Osmosis Membranes to Individual

Cleaning Steps of a Typical Clean-in-Place Protocol." *Journal of Dairy Science* 96(10): 6213–22.

- Bayoumi, Mohamed A., Rania M. Kamal, Salah F. Abd El Aal, and Esmat I. Awad. 2012. "Assessment of a Regulatory Sanitization Process in Egyptian Dairy Plants in Regard to the Adherence of Some Food-Borne Pathogens and Their Biofilms." *International Journal of Food Microbiology* 158(3): 225–31.
- Berkenpas, E., P. Millard, and M. Pereira da Cunha. 2006. "Detection of Escherichia Coli O157:H7 with Langasite Pure Shear Horizontal Surface Acoustic Wave Sensors." *Biosensors and Bioelectronics* 21(12): 2255–62.
- Beyth, Nurit et al. 2015. "Alternative Antimicrobial Approach: Nano-Antimicrobial Materials." *Evidence-Based Complementary and Alternative Medicine* 2015(1): 246012. https://onlinelibrary.wiley.com/doi/full/10.1155/2015/246012.
- Bjarnsholt, Thomas et al. 2013a. "Applying Insights from Biofilm Biology to Drug Development - Can a New Approach Be Developed?" *Nature reviews. Drug discovery* 12(10): 791–808. https://pubmed.ncbi.nlm.nih.gov/24080700/.
- Bjarnsholt, Thomas et al. 2013a. "Applying Insights from Biofilm Biology to Drug Development - Can a New Approach Be Developed?" Nature reviews. Drug discovery 12(10): 791–808. https://pubmed.ncbi.nlm.nih.gov/24080700/.
- Boels, Gauthier. 2011. "Enzymatic Removal of Biofilms: A Report." *Virulence* 2(5): 490–489. https://www.tandfonline.com/doi/abs/10.4161/viru.2.5.17317.
- Bottone, Edward J. 2010. "Bacillus Cereus, a Volatile Human Pathogen." *Clinical Microbiology Reviews* 23(2): 382–98. https://journals.asm.org/doi/10.1128/cmr.00073-09.
- Brown, Helen L. et al. 2015. "Campylobacter Jejuni Biofilms Contain Extracellular DNA and Are Sensitive to DNase I Treatment." *Frontiers in Microbiology* 6(JUL): 699. /pmc/articles/PMC4498105/.
- Büttner, Henning, Dietrich Mack, and Holger Rohde. 2015. "Structural Basis of Staphylococcus Epidermidis Biofilm Formation: Mechanisms and Molecular Interactions." *Frontiers in Cellular and Infection Microbiology* 5(FEB). /pmc/articles/PMC4330918/ (August 27, 2024).
- Carrascosa, Conrado et al. 2021. "Microbial Biofilms in the Food Industry—A Comprehensive Review." *International Journal of Environmental Research and Public Health 2021, Vol. 18, Page 2014* 18(4): 2014. https://www.mdpi.com/1660-4601/18/4/2014/htm.
- Carter, Michelle Qiu et al. 2016. "Curli Fimbriae Are Conditionally Required in Escherichia Coli O157:H7 for Initial Attachment and Biofilm Formation." *Food Microbiology* 57: 81–89.
- Ceri, H. et al. 1999. "The Calgary Biofilm Device: New Technology for Rapid Determination of Antibiotic Susceptibilities of Bacterial Biofilms." *Journal of Clinical Microbiology* 37(6): 1771–76. https://journals.asm.org/doi/10.1128/jcm.37.6.1771-1776.1999.
- Chaitiemwong, N., W. C. Hazeleger, and R. R. Beumer. 2014. "Inactivation of Listeria Monocytogenes by Disinfectants and Bacteriophages in Suspension and Stainless Steel Carrier Tests." *Journal of Food Protection* 77(12): 2012–20.
- Chia, T. W.R. et al. 2009. "Attachment of Different Salmonella Serovars to Materials Commonly Used in a Poultry Processing Plant." *Food microbiology* 26(8): 853–59. https://pubmed.ncbi.nlm.nih.gov/19835771/.
- Chlebicz, Agnieszka, and Katarzyna Śliżewska. 2018. "Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Foodborne Diseases: A Review." *International Journal of Environmental Research and Public Health 2018, Vol. 15, Page 863* 15(5): 863. https://www.mdpi.com/1660-4601/15/5/863/htm.
- Chylkova, Tereza, Myrna Cadena, Aura Ferreiro, and Maurice Pitesky. 2017. "Susceptibility of Salmonella Biofilm and Planktonic Bacteria to Common Disinfectant Agents Used in Poultry Processing." *Journal of food protection* 80(7): 1072–79. https://pubmed.ncbi.nlm.nih.gov/28561639/.
- Corcoran, M. et al. 2014. "Commonly Used Disinfectants Fail to Eradicate Salmonella Enterica Biofilms from Food Contact Surface Materials." *Applied and environmental microbiology* 80(4): 1507–14. https://pubmed.ncbi.nlm.nih.gov/24362427/.
- Coronel-León, J., A. M. Marqués, J. Bastida, and A. Manresa. 2016. "Optimizing the Production of the Biosurfactant Lichenysin and Its Application in Biofilm Control." *Journal of Applied Microbiology* 120(1): 99–111. https://dx.doi.org/10.1111/jam.12992.
- Costerton, J. W., Philip S. Stewart, and E. P. Greenberg. 1999. "Bacterial Biofilms: A Common Cause of Persistent Infections." *Science* 284(5418): 1318–22. https://www.science.org/doi/10.1126/science.284.5418.1318.
- Coughlan, Laura M., Paul D. Cotter, Colin Hill, and Avelino Alvarez-Ordóñez. 2016. "New Weapons to Fight Old Enemies: Novel Strategies for the (Bio)Control of Bacterial Biofilms in the Food Industry." *Frontiers in Microbiology* 7(OCT): 194857. www.frontiersin.org.
- Fagerlund, Annette et al. 2017. "Cleaning and Disinfection of Biofilms Composed of Listeria Monocytogenes and Background Microbiota from Meat Processing Surfaces." *Applied and environmental microbiology* 83(17). https://pubmed.ncbi.nlm.nih.gov/28667108/.
- Fister, Susanne et al. 2016. "Influence of Environmental Factors on Phage-Bacteria Interaction and on the Efficacy and Infectivity of Phage P100." *Frontiers in Microbiology* 7(JUL): 197487. www.frontiersin.org.
- Funari, Riccardo et al. 2018. "Nanoplasmonics for Real-Time and Label-Free Monitoring of Microbial Biofilm Formation." *ACS Sensors* 3(8): 1499–1509. https://pubs.acs.org/doi/full/10.1021/acssensors.8b00287.
- Funari, Riccardo, and Amy Q. Shen. 2022. "Detection and Characterization of Bacterial Biofilms and Biofilm-Based Sensors." *ACS Sensors* 7(2): 347–57. https://pubs.acs.org/doi/full/10.1021/acssensors.1c02722.
- Galié, Serena et al. 2018a. "Biofilms in the Food Industry: Health Aspects and Control Methods." *Frontiers in Microbiology* 9(MAY): 315815. www.frontiersin.org.
- Galié, Serena et al. 2018b. "Biofilms in the Food Industry: Health Aspects and Control Methods." *Frontiers in Microbiology* 9(MAY): 315815. www.frontiersin.org.
- Giaouris, Efstathios, Nikos Chorianopoulos, Agapi Doulgeraki, and George John Nychas. 2013. "Co-Culture with Listeria Monocytogenes within a Dual-Species Biofilm Community Strongly Increases Resistance of Pseudomonas Putida to Benzalkonium Chloride." *PLoS ONE* 8(10). /pmc/articles/PMC3795059/.
- Goeres, Darla M. et al. 2005. "Statistical Assessment of a Laboratory Method for Growing Biofilms." *Microbiology* 151(3): 757–62. https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.27709-0.
- Gomes, L. C., J. Deschamps, R. Briandet, and F. J. Mergulhão. 2018. "Impact of Modified Diamond-like Carbon Coatings on the Spatial Organization and Disinfection of Mixed-Biofilms Composed of Escherichia Coli and Pantoea Agglomerans Industrial Isolates." *International journal of food microbiology* 277: 74–82. https://pubmed.ncbi.nlm.nih.gov/29689455/.
- Gould, L. Hannah et al. 2013. "Increased Recognition of

Non-O157 Shiga Toxin–Producing Escherichia Coli Infections in the United States During 2000–2010: Epidemiologic Features and Comparison with E. Coli O157 Infections." *https://home.liebertpub.com/fpd* 10(5): 453–60. https://www.liebertpub.com/doi/10.1089/fpd.2012.1401.

- Grigore-Gurgu, Leontina et al. 2019. "Biofilms Formed by Pathogens in Food and Food Processing Environments." *Bacterial Biofilms*. https://www.intechopen.com/chapters/70036.
- Gupta, Priya et al. 2015. "Biofilm, Pathogenesis and Prevention—a Journey to Break the Wall: A Review." *Archives of Microbiology 2015 198:1* 198(1): 1–15. https://link.springer.com/article/10.1007/s00203-015-1148-6.
- Gutiérrez, Diana et al. 2014. "Effective Removal of Staphylococcal Biofilms by the Endolysin LysH5." *PLOS ONE* 9(9): e107307. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0107307.
- Han, Qiao et al. 2017. "Removal of Foodborne Pathogen Biofilms by Acidic Electrolyzed Water." *Frontiers in Microbiology* 8(JUN): 251296. www.frontiersin.org.
- Houry, A., R. Briandet, S. Aymerlch, and M. Gohar. 2010. "Involvement of Motility and Flagella in Bacillus Cereus Biofilm Formation." *Microbiology* 156(4): 1009–18.
- Huang, Hui et al. 2014. "Aging Biofilm from a Full-Scale Moving Bed Biofilm Reactor: Characterization and Enzymatic Treatment Study." *Bioresource Technology* 154: 122–30.
- Huang, Kang, Lynne A. McLandsborough, and Julie M. Goddard. 2016. "Adhesion and Removal Kinetics of Bacillus Cereus Biofilms on Ni-PTFE Modified Stainless Steel." *Biofouling* 32(5): 523–33. https://pubmed.ncbi.nlm.nih.gov/27020838/.
- Huis In't Veld, Jos H.J.H.I. 1996. "Microbial and Biochemical Spoilage of Foods: An Overview." *International Journal of Food Microbiology* 33(1): 1–18.
- Iacumin, Lucilla, Marisa Manzano, and Giuseppe Comi. 2016. "Phage Inactivation of Listeria Monocytogenes on San Daniele Dry-Cured Ham and Elimination of Biofilms from Equipment and Working Environments." *Microorganisms 2016, Vol. 4, Page 4* 4(1): 4. https://www.mdpi.com/2076-2607/4/1/4/htm.
- Joo, Hwang Soo, and Michael Otto. 2012. "Molecular Basis of In Vivo Biofilm Formation by Bacterial Pathogens." *Chemistry & Biology* 19(12): 1503–13. http://www.cell.com/article/S1074552112004231/fulltext.
- Kharazmi, Arsalan, Birgit Giwercman, and Niels Høiby. 1999. "[16] Robbins Device in Biofilm Research." *Methods in Enzymology* 310: 207–15.
- Kim, Minyoung Kevin et al. 2017. "Surface-Attached Molecules Control Staphylococcus Aureus Quorum Sensing and Biofilm Development." *Nature Microbiology 2017 2:8* 2(8): 1–12. https://www.nature.com/articles/nmicrobiol201780.
- Klančnik, Anja et al. 2020. "Anti-Adhesion Activity of Phytochemicals to Prevent Campylobacter Jejuni Biofilm Formation on Abiotic Surfaces." *Phytochemistry Reviews 2020 20:1* 20(1): 55–84. https://link.springer.com/article/10.1007/s11101-020-09669-6.
- Korber, D. R., A. K. Mangalappalli-Illathu, and S. Vidovic'. 2009. "Biofilm Formation by Food Spoilage Microorganisms in Food Processing Environments." *Biofilms in the Food and Beverage Industries*: 169–99.
- Langsrud, S. 2015. "Bio ▫ Lms Listeria Monocytogenes : Bio ▫ Lm Formation and Persistence in Food- Listeria Monocytogenes : Biofilm Formation and Persistence in Food-Processing Environments." (September 2004): 107–21.
- Lee, Jintae et al. 2007. "Enterohemorrhagic Escherichia Coli Biofilms Are Inhibited by 7-Hydroxyindole and Stimulated by Isatin." *Applied and Environmental Microbiology* 73(13): 4100–4109. https://journals.asm.org/doi/10.1128/AEM.00360-07.
- Leung, J. W. et al. 1998. "Is There a Synergistic Effect between Mixed Bacterial Infection in Biofilm Formation on Biliary Stents?" *Gastrointestinal Endoscopy* 48(3): 250–57.
- Lu, Timothy K., and James J. Collins. 2007. "Dispersing Biofilms with Engineered Enzymatic Bacteriophage." *Proceedings of the National Academy of Sciences of the United States of America* 104(27): 11197–202. https://www.pnas.org/doi/abs/10.1073/pnas.0704624104.
- Mattana, S. et al. 2017. "High-Contrast Brillouin and Raman Micro-Spectroscopy for Simultaneous Mechanical and Chemical Investigation of Microbial Biofilms." *Biophysical Chemistry* 229: 123–29.
- McKenney, David et al. 1998. "The Ica Locus of Staphylococcus Epidermidis Encodes Production of the Capsular Polysaccharide/Adhesin." *Infection and Immunity* 66(10): 4711–20. https://journals.asm.org/doi/10.1128/iai.66.10.4711-4720.1998.
- Meireles, Ana, Anabela Borges, Efstathios Giaouris, and Manuel Simões. 2016. "The Current Knowledge on the Application of Anti-Biofilm Enzymes in the Food Industry." *Food Research International* 86: 140–46.
- Mizan, Md Furkanur Rahaman, Iqbal Kabir Jahid, and Sang Do Ha. 2015. "Microbial Biofilms in Seafood: A Food-Hygiene Challenge." *Food Microbiology* 49: 41–55.
- Morris, Rachel L., and Thomas M. Schmidt. 2013. "Shallow Breathing: Bacterial Life at Low O(2)." *Nature reviews. Microbiology* 11(3): 205–12. https://pubmed.ncbi.nlm.nih.gov/23411864/.
- Nam, Hyegyeong et al. 2014. "Efficacy of Gaseous Chlorine Dioxide in Inactivating Bacillus Cereus Spores Attached to and in a Biofilm on Stainless Steel." *International Journal of Food Microbiology* 188: 122–27.
- Olanbiwoninu, A. A., and B. M. Popoola. 2023. "Biofilms and Their Impact on the Food Industry." *Saudi Journal of Biological Sciences* 30(2): 103523.
- Paradh, A. D., W. J. Mitchell, and A. E. Hill. 2011. "Occurrence of Pectinatus and Megasphaera in the Major UK Breweries." *Journal of the Institute of Brewing* 117(4): 498–506. https://onlinelibrary.wiley.com/doi/full/10.1002/j.2050-0416.2011.tb00497.x.
- Parsek, Matthew R., and E. P. Greenberg. 2005. "Sociomicrobiology: The Connections between Quorum Sensing and Biofilms." *Trends in Microbiology* 13(1): 27–33. http://www.cell.com/article/S0966842X04002616/fulltext.
- Parvinzadeh Gashti, Mazeyar et al. 2016. "A Microfluidic Platform with PH Imaging for Chemical and Hydrodynamic Stimulation of Intact Oral Biofilms." *Lab on a Chip* 16(8): 1412–19. https://pubs.rsc.org/en/content/articlehtml/2016/lc/c5lc01540e.
- Pires, Diana P. et al. 2016. "Bacteriophage-Encoded Depolymerases: Their Diversity and Biotechnological Applications." *Applied Microbiology and Biotechnology 2016 100:5* 100(5): 2141–51. https://link.springer.com/article/10.1007/s00253-015-7247-0 .
- Pousti, Mohammad et al. 2018. "Microfluidic Bioanalytical Flow Cells for Biofilm Studies: A Review." *Analyst* 144(1): 68–86. https://pubs.rsc.org/en/content/articlehtml/2019/an/c8an01526k (August 28, 2024).
- Puligundla, P., and C. Mok. 2017. "Potential Applications of Nonthermal Plasmas against Biofilm-

Associated Micro-Organisms in Vitro." *Journal of applied microbiology* 122(5): 1134–48. https://pubmed.ncbi.nlm.nih.gov/28106311/.

- Rothrock, Michael J. et al. 2017. "Listeria Occurrence in Poultry Flocks: Detection and Potential Implications." *Frontiers in Veterinary Science* 4(AUG): 269657. www.frontiersin.org.
- Sadiq, Faizan A. et al. 2017. "Propensity for Biofilm Formation by Aerobic Mesophilic and Thermophilic Spore Forming Bacteria Isolated from Chinese Milk Powders." *International Journal of Food Microbiology* 262: 89–98.
- Sharma, Satish et al. 2023. "Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment." *Microorganisms* 11(6). /pmc/articles/PMC10305407/.
- Shen, Yang, Thomas Köller, Bernd Kreikemeyer, and Daniel C. Nelson. 2013. "Rapid Degradation of Streptococcus Pyogenes Biofilms by PlyC, a Bacteriophage-Encoded Endolysin." *Journal of Antimicrobial Chemotherapy* 68(8): 1818–24. https://dx.doi.org/10.1093/jac/dkt104 .
- Speziale, Pietro, and Joan A. Geoghegan. 2015. "Biofilm Formation by Staphylococci and Streptococci: Structural, Functional, and Regulatory Aspects and Implications for Pathogenesis." *Frontiers in Cellular and Infection Microbiology* 5(APR): 144309. www.frontiersin.org.
- Sprung, C. et al. 2009. "Detection and Monitoring of Biofilm Formation in Water Treatment Systems by Quartz Crystal Microbalance Sensors." *Water Science and Technology* 59(3): 543–48.
- Srey, Sokunrotanak, Iqbal Kabir Jahid, and Sang Do Ha. 2013. "Biofilm Formation in Food Industries: A Food Safety Concern." *Food Control* 31(2): 572–85.
- Torres, Claudia Esperanza et al. 2011. "Enzymatic Treatment for Preventing Biofilm Formation in the Paper Industry." *Applied Microbiology and Biotechnology* 92(1): 95–103. https://link.springer.com/article/10.1007/s00253-011-3305-4.
- Vacheethasanee, Katanchalee, and Roger E Marchant. 2000. "Factors Influencing Bacterial Adhesion" eds. Yuehuei H An and Richard J Friedman. *Handbook of Bacterial Adhesion: Principles, Methods, and Applications*: 53–72. https://link.springer.com/chapter/10.1007/978-1-59259-224-1_4 (August 27, 2024).
- Varga, László, and Jeno Szigeti. 2016. "Use of Ozone in the Dairy Industry: A Review." *International Journal of Dairy Technology* 69(2): 157–68. https://onlinelibrary.wiley.com/doi/full/10.1111/1471-0307.12302.
- Veerachamy, Suganthan, Tejasri Yarlagadda, Geetha Manivasagam, and Prasad Kdv Yarlagadda. 2014. "Bacterial Adherence and Biofilm Formation on Medical Implants: A Review." *http://dx.doi.org/10.1177/0954411914556137* 228(10): 1083–99. https://journals.sagepub.com/doi/abs/10.1177/0954411914556137.
- Vestby, L. K. et al. 2014. "Synthetic Brominated Furanone F202 Prevents Biofilm Formation by Potentially Human Pathogenic Escherichia Coli O103:H2 and Salmonella Ser. Agona on Abiotic Surfaces." *Journal of Applied Microbiology* 116(2): 258–68. https://dx.doi.org/10.1111/jam.12355.
- Villain-Simonnet, Agnès, Michel Milas, and Marguerite Rinaudo. 2000. "A New Bacterial Exopolysaccharide (YAS34). II. Influence of Thermal Treatments on the Conformation and Structure. Relation with Gelation Ability." *International journal of biological macromolecules* 27(1): 77–87. https://pubmed.ncbi.nlm.nih.gov/10704989/.
- Wang, Huhu et al. 2016. "Removal of Salmonella Biofilm Formed under Meat Processing Environment by Surfactant in Combination with Bio-Enzyme." *LWT - Food Science and Technology* 66: 298–304.
- Waszczuk, K. et al. 2012. "Evaluation of Pseudomonas Aeruginosa Biofilm Formation Using Piezoelectric Tuning Fork Mass Sensors." *Sensors and Actuators B: Chemical* 170: 7–12.
- Whitehead, K. A., & Verran, J. (2015). Formation, architecture and functionality of microbial biofilms in the food industry. Current Opinion in Food Science, 2, 84-91. http://dx.doi.org/10.1016/j.cofs.2015.02.003
- Wu, Ping et al. 2019. "Feasibility Study on Direct Fermentation of Soybean Meal by Bacillus Stearothermophilus under Non-Sterile Conditions." *Journal of the Science of Food and Agriculture* 99(7): 3291–98. https://onlinelibrary.wiley.com/doi/full/10.1002/jsfa.9542 (September 10, 2024).
- Yang, Liang, and Michael Givskov. 2015. "Chemical Biology Strategies for Biofilm Control." *Microbiology spectrum* 3(4). https://pubmed.ncbi.nlm.nih.gov/26350311/.
- Yang, Yishan et al. 2016. "Biofilm Formation of Salmonella Enteritidis under Food-Related Environmental Stress Conditions and Its Subsequent Resistance to Chlorine Treatment." *Food Microbiology* 54: 98–105.
- Zhao, X., Zhao, F., Wang, J., & Zhong, N. (2017). Biofilm formation and control strategies of foodborne pathogens: food safety perspectives. RSC advances, 7(58), 36670-36683.