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REVIEW

Bacterial biofilm formation on stainless steel in the food processing environment and its health implications

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Abstract

Biofilm formation (BF) and production in the food processing industry (FPI) is a continual threat to food safety and quality. Various bacterial pathogens possess the ability to adhere and produce biofilms on stainless steel (SS) in the FPI due to flagella, curli, pili, fimbrial adhesins, extra polymeric substances, and surface proteins. The facilitating environmental conditions (temperature, pressure, variations in climatic conditions), SS properties (surface energy, hydrophobicity, surface roughness, topography), type of raw food materials, pre-processing, and processing conditions play a significant role in the enhancement of bacterial adhesion and favorable condition for BF. Furthermore, biofilm formers can tolerate different sanitizers and cleaning agents due to the constituents, concentration, contact time, bacterial cluster distribution, and composition of bacteria within the biofilm. Also, bacterial biofilms' ability to produce various endotoxins and exotoxins when consumed cause food infections and intoxications with serious health implications. It is thus crucial to understand BF's repercussions and develop effective interventions against these phenomena that make persistent pathogens difficult to remove in the food processing environment.

Keywords Biofilm formation · Food processing industry · Stainless steel · Sanitizers · Health implications

Introduction

Bacterial adhesion to stainless steel (SS) has become an emerging challenge in the food industry. It begins with an initial physical attraction of bacteria to the substrate followed by cell multiplication, resulting in biofilm development stages until the cellular mass is thick enough to aggregate nutrients, residues, and other microorganisms (Garrett et al. 2008). A biofilm is a structured and functional consortium of single or multiple species embedded in a self-produced organic polymer matrix and carbohydrate-binding substances adherent to an abiotic or biotic contact surface (Kawakami et al. 2010). The development of bacterial biofilms on SS in the food processing industry can be a source of survival for pathogenic microorganisms and numerous spoilage, leading to food contamination, thereby compromising food safety and shelf-life. Figure 1 shows the interplay of factors that enhance bacterial adherence and possible biofilm formation (BF) in food during supply, processing, and production.

In the early stages of BF, some bacterial cell surface properties like hydrophobicity, presence of an S-layer, and electrostatic repulsion or attraction usually contribute to adhesion development to surfaces, thus increasing chemical communication between cells, accumulation of nutrients for metabolic use, and the production of enzymes that degrade antimicrobial substances that reduce growth and influence communities' organization (Colagiorgi et al. 2017; Renner and Weibel 2011). The nature of the contact surface, genera/species and strain composition, and biotic and abiotic conditions determine the BF progress to a complex matrix structure (Armbruster and Parsek 2018). The attachment of cells and progress in the production of biofilm is shown in Fig. 2.

Stainless steel (SS) is a family of corrosion- and heat-resistant iron-based alloys containing various compounds such as chromium, nickel, and molybdenum (austenitic SS); chromium and carbon (ferritic SS); chromium, carbon, molybdenum, titanium, and nitrogen (Martensitic SS); and

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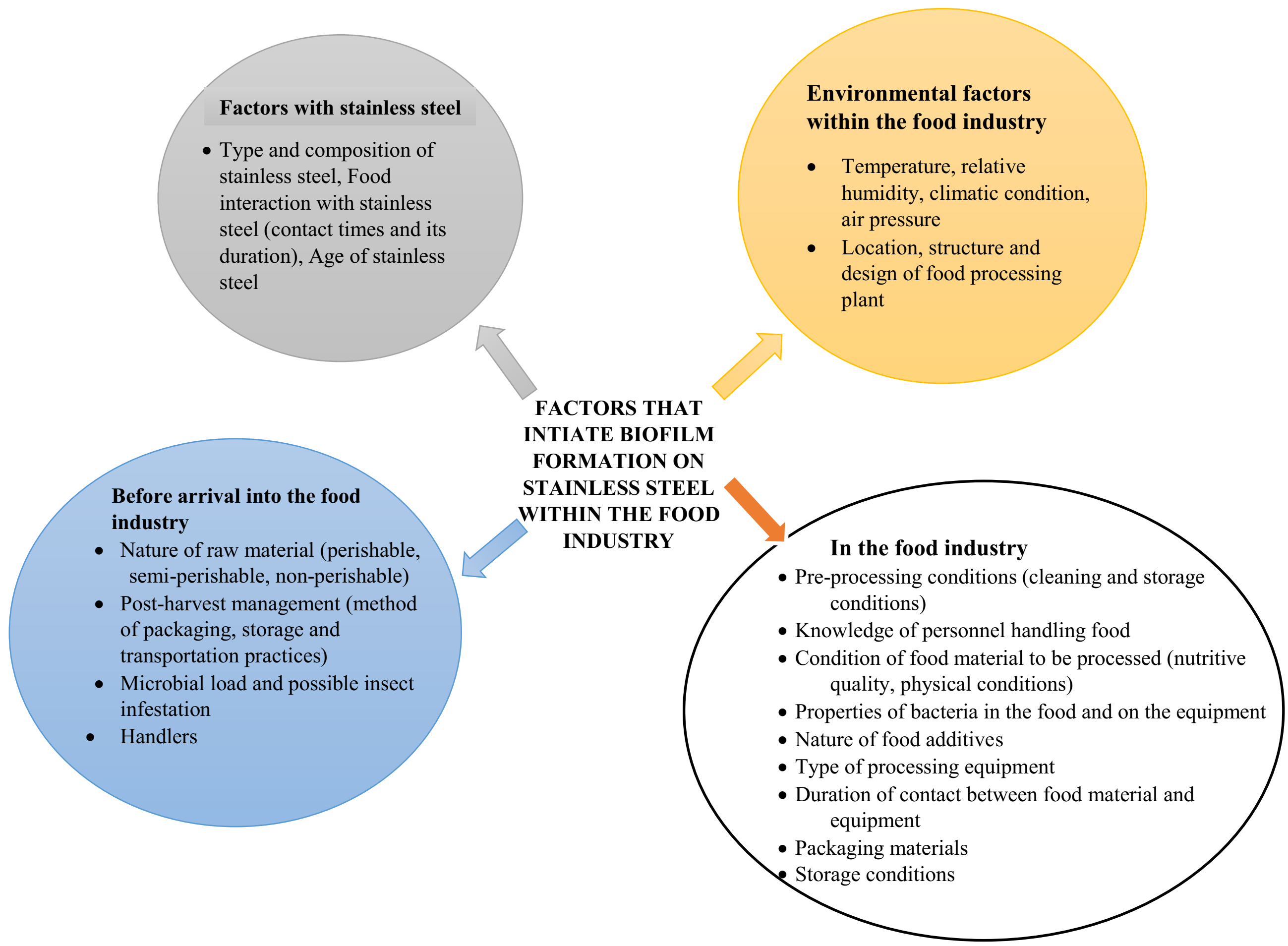

Fig. 1 Factors that influence the initiation of biofilm on stainless steel in the food industry during processing and production

the duplex SS, which have a mixture of austenitic and ferritic component (Decléty 2003). Some aerobic and anaerobic bacteria can adapt to the SS environment. They create unequal aeration zones or a more hostile environment to enhance its survival and damage SS by acting in an isolated or symbiotic manner with the production of metabolites that lead to a local break that causes passive and passivating layer favoring corrosion (Ibars et al. 1992).

Adhesion and subsequent BF on SS under this circumstance greatly influence the intrinsic and extrinsic factors of food, the composition of steel, type and properties of microorganism(s) involved, and the biotic and abiotic factors in the food processing industry. The associated-biofilm may increase the organisms' pathogenicity, corrosion of contact-metal surfaces, alter organoleptic properties of food products, and lead to serious health problems. This review summarizes these factors' interplay in the food processing and production environment and its possible health consequences.

Properties of Stainless steel (SS) that facilitates bacterial adhesion

The general iron corrosion mechanisms include chemical and microbiological induced corrosion (MIC), whose source of electrons originate from a hydrogen film on the metal surface. However, corrosion and crust formation stop upon loss of direct metal contact. Besides, the electron source of electrical MIC can be directly extracted from iron to produce sulfuric corrosion crust, enabling the transfer of electrons to the microbes for growth (Kip and van-Veen 2015). As a component of some SS, nickel can also tolerate biofilms in a time-dependent manner by increased adherence during the period of early cell adaptation to sub-inhibitory concentrations of nickel, leading to increased tolerance and formation of very thick biofilms (Perrin et al. 2009). Formation of complex microbial biofilm communities can also occur on SS with minor manganese concentrations in flow-through systems (Kielemoes et al. 2002).

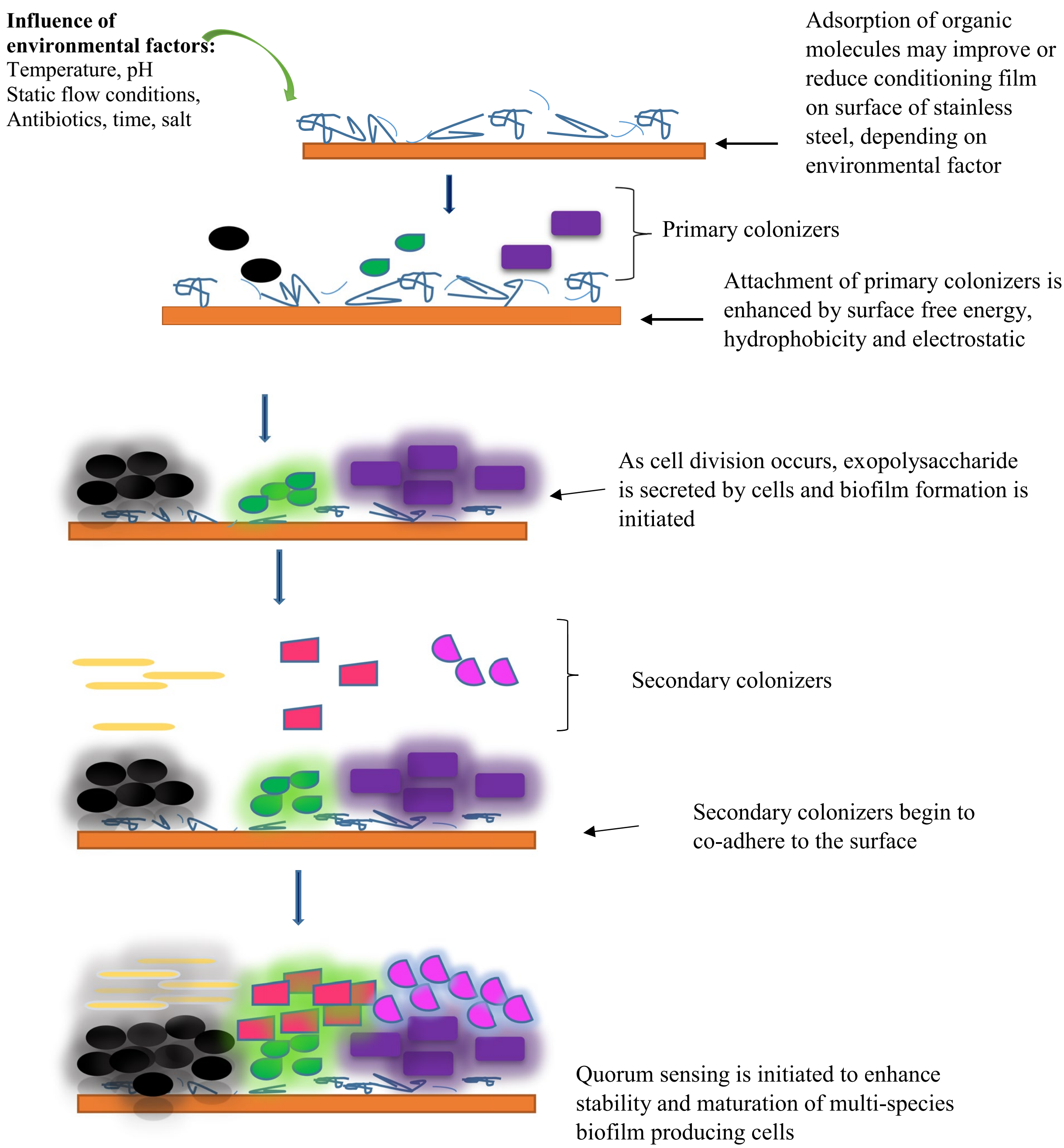

Fig. 2 Attachment of cells to stainless steel surfaces and progression in the development of biofilm

MIC of steel varies depending on the approach (aerobic and anaerobic corrosion), which may occur in combination with other corrosion failures and is known to induce a localized attack, including de-alloying, pitting, localized galvanic corrosion, and stress corrosion cracking (Kiani-Khouzani et al. 2019). Bio-corrosion of SS can be caused by iron-reducing bacteria (IRB), iron- and manganese-oxidizing bacteria, acid-producing bacteria, and sulfate-reducing bacteria (SRB), sulfur-oxidizing bacteria, iron oxidizers, iron reducers, and manganese oxidizers that secrete organic acids and produce extracellular polymeric substances (Kip and van-Veen 2015), destroying the passive film of stainless through the formation of different sulfide, subsequently resulting in pitting corrosion (Liu et al. 2018; Kiani-Khouzani et al. 2019). Fe (III) minerals can be microbiologically reduced across bacteria and archaea domains by strict anaerobic or facultative Fe-reducing bacteria using a wide range of organic compounds as electron donors or H_2 (Luef et al. 2013).

In anaerobic environments, IRB reduces ferric ions as a final electron acceptor for the anaerobic decomposition of organic compounds as a key parameter for metabolic assays. However, its ability to use organic electron donors reduces significantly, while some IRB such as *Shewanella putrefaciens*, *Shewanella algae*, and *Pseudomonas* spp. can use a wide variety of electron acceptors such as oxygen (Ebrahiminezhad et al. 2017). Under micro-aerobic conditions where ferrous ions can potentially accumulate with low sulfide ion concentration, a combination of IRB, SRB, and fermentative bacteria increases steel corrosion, possibly through de-stabilization of the protective sulfide film (Valencia-Cantero and Peña-Cabriales 2014). The mechanism likely to enhance corrosion in some carbon-containing SS is the de-stabilization and dissolution of the passivating magnetite layer by reducing structural Fe (III) coupled to H_2 oxidation (Schütz et al. 2015).

The interactions between the SS surface, abiotic corrosion products, and bacterial cells and their metabolic products increase the corrosion damage degree of the passive film and accelerated pitting propagation (Xu et al. 2006). When the surface energy becomes lower in some SS (for example, 304), bacterial biofilm adhesion may be weaker due to changes in surface functionalities of SS after thermal treatment, which impacts the adhesion nature as it influences the contact angle and surface free energy (Nan et al. 2015). Also, hydrophobicity and surface roughness has a significant role in bacterial adhesion. Thus, a less hydrophobic SS surface attracts more bacteria than more hydrophobic surfaces (Jindal et al. 2016). The physicochemical aspects' influence consists of surface wettability, tension, topography, and charge of the substratum surface on bacterial attachment. High free energy, an inter-facial property of a surface, and wet surfaces promote bacterial adhesion (Boulange-Petermann et al. 1993). Some other bacterial cell adhesion approaches to SS surfaces could also result from Brownian motion, sedimentation, movement with the liquid flow, bacterial motility with cell surface appendages, and interaction with other cells to form aggregates (Teughels et al. 2006).

Surface irregularities also enhance bacterial settling and adhesion because surface roughness higher than 0.2 µm increases the degree of bacterial adhesion, particularly in SS containing titanium, titanium nitride, fluorine modified hydroxyapatite, and zinc modified fluorine modified hydroxyapatite thin films (Jeyachandran et al. 2007). Surface charge is also a factor that promotes bacterial attachment. A high amount of PO_4, NH_2, and COOH groups make most bacteria cells possess a negative surface charge, which hinders bacterial attachment. In contrast, a positively charged surface encourages bacterial cell adhesion to the surface (Mediaswanti 2016). Metal-oxides can increase the adhesion of negatively charged bacteria to surfaces primarily due to their positive charge. However, the hydrophobicity of a metal-oxide surface can also increase bacteria's adhesion (Li and Logan 2004).

Surface properties of microorganism that enhance adhesion on SS

The importance of bacteria flagella-driven motility, chemotaxis, extracellular polymeric substances (EPS), surface proteins, and their metabolic activity are important bacterial adhesion elements. It determines the integrality and compactness of biofilm, resulting in the pitting corrosion process, elevated corrosion damage of the passive film, and accelerated pitting corrosion (Zhang et al. 2007). The nature of bacteria and their serotype also determines the extent and strength of adhesion and BF. Bacterial adherence promotes the development of biofilm in cells. As this course progresses, a quasi-dormant state is produced that increases biocide resistance and biofilm cells can sense and actively respond to the biocide challenge by deploying defensive stress responses, triggering unpleasant changes in food quality (Chmielewski and Frank, 2003).

The extra polymeric substances comprising of polysaccharides, proteins, and nucleic acids are responsible for the biofilm structure in terms of the morphology, structure, cohesion, and functional integrity of the biofilm (Grigore-Gurgu et al. 2019). Curli genes promote the BF when bacteria encounter sub-inhibitory nickel concentrations in the surrounding medium (Perrin et al. 2009). The adhesion effect of cell surface hydrophobicity (CSH) and fimbriae production in some bacteria are temperature-dependent. The high temperature increased the CHS level, which correlates with BF in Shiga toxin-producing *Escherichia coli* (STEC) isolates. Conversely, there was no fimbriae production in *Salmonella* at temperatures below 20 °C (Ma et al. 2019). CHS and the presence of extracellular filamentous appendages, such as pili and flagella, can influence the rate and degree of attachment (Meliani and Bensoltane 2015). When several bacteria are involved in BF, cell-to-cell communication is vital to reach the required microbial cell density, thus, leading to the secretion of signaling molecules, known as auto-inducers, facilitating quorum sensing (QS) (Jamal et al. 2018). QS has been implicated in the production of virulence factors and biofilm formation by foodborne pathogens. In response to stressful external conditions like cleaning and disinfection procedures, these pathogens secrete EPS, extracellular proteases, perform swimming and swarming motility, and other physiological function. This enables the release of enzymes, heat production in some cases that degrade food and subsequently leading to spoilage (Machado et al. 2020). Depending on the composition of biofilm-forming bacteria (motile cells, matrix producers, and sporulating cell), environmental temperature, processing techniques, and type of SS used in the equipment design, QS between related bacteria, lateral gene transfer, and environmental response increases the persistence of vegetative forms, which favors their complex exopolysaccharide, protein, and extracellular DNA matrix (Galié et al. 2018; Aijuka and Buys 2019). Food quality is compromised because some biofilm cells release stable substances and subsequently contaminate food, resulting in foodborne disease transmission.

Effect of environmental/industrial factors on biofilm formation

BF in FPI and their corrosive ability vary depending on the type of microorganism, type of food, processing conditions, environmental factors (temperature, atmospheric pressure), incubation time, and SS type. A moderate to strong biofilms of STEC can be formed on SS at 22 °C, while low-temperature environments (13 °C) reduce BF on food contact surfaces (Ma et al. 2019). The rate of adhered bacteria increases with an increase in surface roughness, numbers of cracks, voids, and gaps (Bohinc et al. 2016).

In the FPI, both static and flow conditions influence the cell density and strength of attachment. Static or low flow conditions aid in the development of isotropic structures, but higher uni-directional flow produces filamentous cells with directionality evidence (Goller and Romeo 2008). The low shear force allows weak rolling adhesion, and cells spread out and colonize more substratum area than high shear stress where cells remain in tight micro-colonies. Consequently, an optimum flow rate allows a stable interaction between bacteria and substrate, reflecting the balance between bacterial delivery rate and the force acting on the attached bacterium and preferred colonization sites (Katsikogianni and Missirlis 2004). Furthermore, a surface-attached bacterium experiences a local force that is normal to the surface in the initial contact (adhesive force). Conversely, in an environment with a flow, the surrounding fluid's viscosity generates a hydrodynamic (shear) force on the cell that is tangential to the surface in the flow direction. Thus, surface motility may produce a friction force tangential to the cell wall and localized at the substrate interface (Persat et al. 2015).

Properties of the food matrix, composition, and concentration could cause bacterial physiological changes related to surface attachment and bacterial adhesion (Katsikogianni and Missirlis 2004). Additionally, food concentration/viscosity and composition also determine the formation of extracellular polymeric substances produced by bacteria in the substratum, which provides anchorage and nutrients to the bacterial community (Shi and Zhu 2009). In some instances, biofilm can be enhanced in a poor nutrient environment rather than a nutrient-rich medium. In addition, the nutritional composition of food may sometimes form residues that can influence the initiation, type, and rate of bacterial adherence (Karatan and Watnick 2009). Different microorganisms have been associated with specific food spoilage, thus leading to adherence of mixed biofilm population, thereby adding more complexity to attachment and biofilm formation (Galié et al. 2018). Additionally, the proliferation and biofilm-forming activity of various pathogenic microorganisms is enhanced with the concentrations of glucose. For example, low glucose concentrations activate biofilm formation by *Bacillus subtilis,* stimulating a positive regulator of biofilm formation (*Spo0A*). In contrast, high concentrations inhibit it by stimulating *CcpA*, which represses a gene that decreases cells' attachment rate (Karatan and Watnick 2009).

Generally, biofilm formation can be influenced by different osmolarities, depending on the osmolyte type. An increase in biofilm production by *B. subtilis* was reported with increased Mn2+ and glycerol concentration, while NaCl addition significantly induced microorganisms growth. Furthermore, D-sorbitol's addition had a greater influence than NaCl on the strains' growth (Kavamura and de Melo 2014). In another study, 100 mM NaCl in growth medium repressed transcription of curli genes by the transcription factor, CpxR. However, the addition of similar sucrose concentrations does not produce the same effect, suggesting the role of environmental signaling on ionic strength (Jubelin et al. 2005). The pH for different biofilm development generally varies between 5.5 and 9.0, while the temperature can range between 4 and 60 °C (Jones et al. 2015; Galié et al. 2018).

Biofilm tolerance to disinfectants and cleaning agents

SS used for food contact surfaces normally contains anticorrosive properties, disinfectants and cleaning agents commonly used to treat food contact surfaces (like peroxides, chloramines, or hypochlorite) can reduce BF. However, the SS surface may not resist the activity of hypochlorite as a cleaning agent but the dominating pH and its percentage in the solution govern its bactericidal activities (Fukuzaki et al. 2007). Chlorine treatments are known to reduce BF. Its disinfection efficacy depends on the cluster size distribution, food sample types, species and serovar composition (Behnke et al. 2011), sanitizer tolerance, and bacterial post-sanitization recovery growth closely associated with strains' biofilm-forming ability (Wang et al. 2017). Additionally, strong biofilm formers can demonstrate durable tolerance to quaternary ammonium chloride (QAC), chlorine dioxide, and multiple antimicrobial agents.

The efficacy of sanitizing agent can be determined by the type of food to be processed, the composition and surface structure of SS, and the sanitize exposure period. Some reports have recommended a combination of sanitizing agents. Carballo and Araújo (2012) reported higher doses of disinfectants (twice to four times of quaternary ammonium compounds, and alquyl-diethylene-diamineglycine and di-alquyldiamineethylglycine) than those endorsed by the manufacturer is needed to completely eliminate planktonic bacteria and an additional application of heat will enhance detachment of bacteria. This suggests that a combination of heat and chemicals for the decontamination of surfaces can present additional security in FPI. In another study from the dairy industry, some bacteria form biofilms during the exponential growth phase at a short contact time of 2 h and exhibit matured stages of the biofilm cycle at 4 h. However, 4% of sanitizing agents (oxisan and chlorine) can efficiently reduce biofilm concentrations up to 82% on SS (Meesilp and Mesil 2019). Therefore, a combination of sanitizers (modified QAC, hydrogen peroxide, and diacetin) achieved about 6–7 log reduction against strong biofilm formers (Aryal and Muriana 2019).

Potential implications of BF on food safety and products

There is concern from consumers, regulatory agencies, and the food industry on the potential adverse effects (toxicity) associated with food development. These may include delivery systems for colors, flavors, preservatives, nutrients, nutraceuticals, or those used to modify the optical, rheological, or flow properties of foods or food packaging (McClements and Xiao 2017). In addition to the genetic predisposition of bacteria to form biofilms, various environmental factors such as temperature, pH, and the growth medium composition or cell and contact surface properties may enhance biofouling (Bezek et al. 2019). Also, biofouling in industrial and drinking water has several harmful effects such as chemical and microbiological destruction of water quality, inducing changes in color, taste, and odor due to release of chemical compounds and, more significantly, a threat to animal and human health resulting in outbreaks (Tasneem et al. 2018). Factors that enhance biofouling are shown in Fig. 3. An effective way to minimize

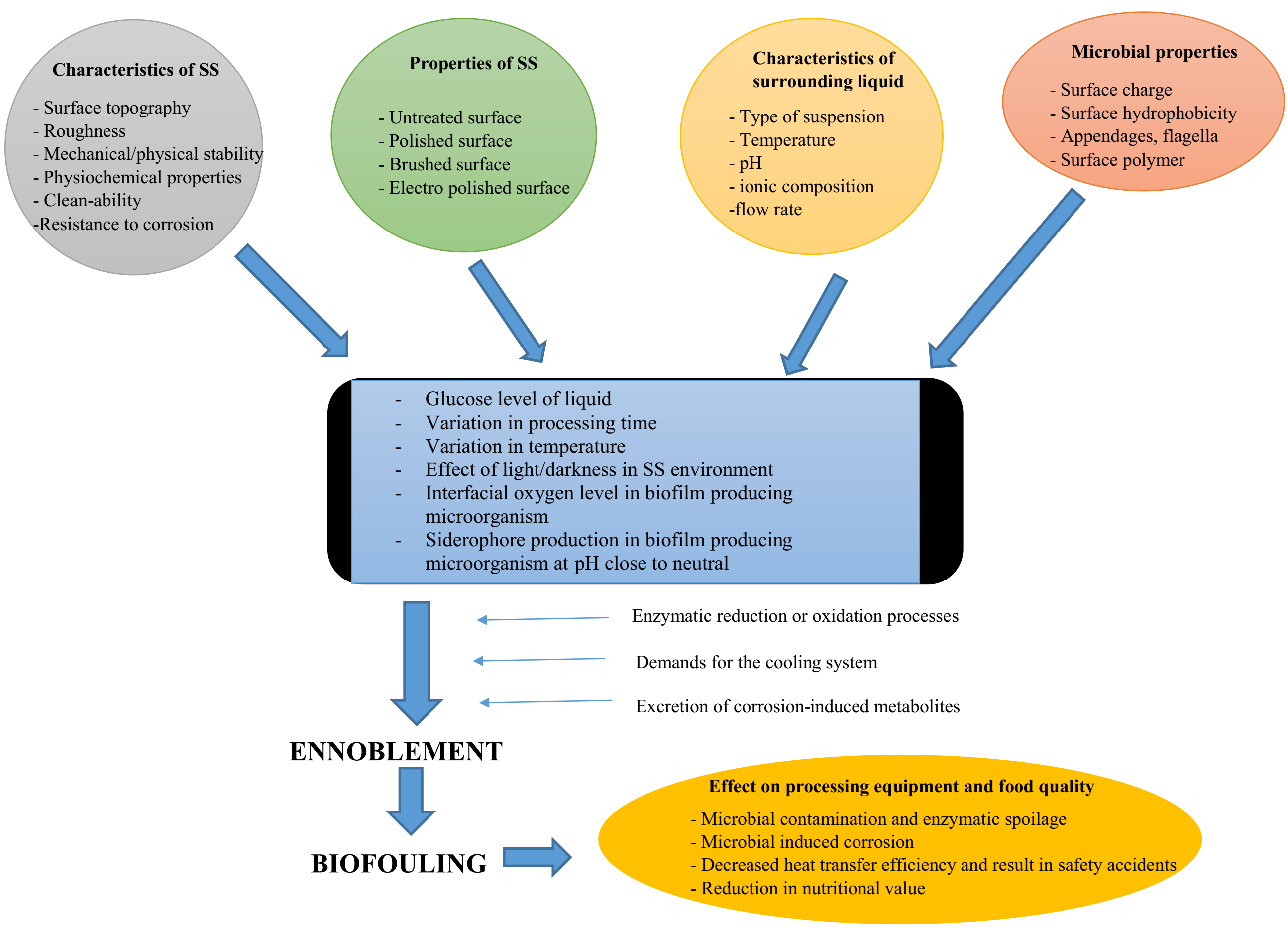

Fig. 3 Factors influencing biofouling and effect on food quality

Table 1 Properties of bacterial biofilms, nature of adherence on stainless steel and implications on human health

Microorganism	Microorganism's property to cause biofilm	Effect in the gut	Nature of adherence to stainless steel	Health implications	References
Listeria monocytogenes	Teichoic acids, exopolysaccharides, surface-associated proteins, and eDNA	Gastroenteritidis, gall bladder infection	Rough SS surfaces that are either moistened or dried	Septicemia, meningitis spontaneous abortion or damage to the fetus	Colagiorgi et al. (2017), Galié et al. (2018), and Gahan and Hill (2005)
Bacillus cereus	Endospore, flagella motility, production exopolysaccharides, proteins and extracellular DNA	diarrheal enterotoxins, emetic toxin	Air–liquid interface and under submerged conditions	Cellular damage and immunomodulatory effects	Galié et al. (2018)
Salmonella spp	Lipopolysaccharide, flagella motility	Endocytosis	Can be developed under any condition but depends on the type of SS finishing	Gastroenteritis/septicemia, depress myocardial function, altered lymphocyte function, septic shock	Schlisselberg and Yaron (2013) and Galié et al. (2018)
Escherichia coli	Flagella and fimbria (type 1 or curli)	Entero-hemorrhagic toxin	Hydrophobicity of the surface material	Gastroenteritis, hemolytic uremic syndrome, with acute kidney injury and thrombocytopenia	Galié et al. (2018)
Pseudomonas spp	Extra polymeric substances, cell surface hydrophobicity, appendages, quorum sensing	extracellular toxins, which include phytotoxic factor, pigments, hydrocyanic acid, proteolytic enzymes, phos-pholipase, enterotoxin, exotoxin, and slime, protein exotoxins	Rougher surfaces, low-energy hydrophobic surfaces	Diarrheal diseases	Zameer et al. (2016) and Galié et al. (2018)
Staphylococcus aureus	Extracellular polymeric substances, polysaccharide intercellular adhesin	Alpha-toxin and leukocidin Beta toxins -	Surface roughness or topography	Inhibit macrophage phagocytosis, induce cytotoxicity, lysis erythrocytes and lymphocytes	Reffuveille et al. (2017), Galié et al. (2018)
Klebsiella	Extracellular polymeric substances	Endotoxins and exotoxins	Component and surface structure, surface roughness, Z-potential, and surface free energy	pneumonia, acute intestinal infections, urogenital infections, conjunctivitis	Kathiresan and Mohan (2017) and Malhotra et al. (2019)
Aeromonas spp.	polar and lateral flagella	cytotoxins and enterotoxins	Survives on any type of SS surface and can proliferate in the presence of residual concentrations of disinfectants	Acute gastroenteritis, bacteremia, pancreatitis, hepatobiliary-tract infections, soft-tissue infections, indwelling-device-related infections, brain abscesses, meningitis, endocarditis, pleuropulmonary infections, peritonitis, and hemolytic-uremic syndrome	Craveiro et al. (2015) and Rhee et al. (2016)

surface biofouling on SS is temperature control (Bezek et al. 2019). The food matrix contamination can also lead to food spoilage, an infection, and individual or multiple intoxications, as seen in Table 1. These may lead to considerable economic losses in the food processing environments as the methodology used for sampling raw materials and processing, processing plants, and even products may be halted/destroyed.

Conclusion

The ubiquitous nature of BF and their contact with food surfaces within the processing industry act as a persistent cause of contamination and risk to microbial safety and quality of food products, resulting in economic losses and numerous foodborne diseases. Although the initial microbial load in the raw material (before production) may play an important role in the development of biofilms in the food processing plant, it is essential to carefully analyze the type of SS material, structure and design, nature of food, duration of food contact with SS, and other extrinsic factors to enable quality control and identify the biofilm-prone zone in the processing lines. The importance of systematic cleaning of food contact surfaces preceding sanitizing strategies and the appropriate selection of sanitizers should also be emphasized.

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Author contribution SD, TAA, and OAI made significant contributions to the idea of this review and wrote the manuscript. All authors read and approved the final manuscript to ensure accuracy and proper report of the work.

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Declarations

Conflict of interest The authors declare no competing interests.

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