Good Hygiene Practices and Their Prevention of Biofilms in the Food Industry

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Cambridge Scholars Publishing

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This book first published 2019

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-3589-4
ISBN (13): 978-1-5275-3589-3

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PREFACE

The food production system is a complex, dynamic, and globally oriented process that has changed significantly in the previous decade. New technologies, consumers' perceptions, and increased awareness of emerging pathogens have forced the food production sector to evaluate the quality and safety of their products. In recent years, there have been significant advances in the understanding of biofilm development as a complex natural phenomenon that eventually occurs on all surfaces. At the same time, improvements in food technology science have been accompanied by massive changes in cleaning and disinfection agents and procedures.

Therefore, the purpose of this book is to provide information needed to manage the biofilm biomass in food facilities at as low a level as possible and to ensure hygienic practices and food safety. To enable the reader to understand pathogenic and spoilage bacteria in the food industry, Chapter 1 is devoted to dominant bacteria in food-processing facilities. There are other microbiological risks related to food safety (e.g., viruses, fungi, and protozoa), but they are not included in the discussion of this book. Chapter 2 presents information on food contact materials with a discussion about common surfaces in the food industry and their properties affecting bacterial adhesion. Furthermore, food contact materials can represent other risks, such as chemical and physical hazards, but that discussion is beyond the aim of this book. Bacterial adhesion and consequent biofilm formation strongly depend on the environmental conditions that bacteria experience and are thus addressed in Chapter 3. Chapter 4 discusses the biofilm life cycle from initial adhesion to growth, maturation, and dispersion. Chapter 5 is about specific conditions in food-processing facilities, such as meat production, and the dairy, beverage, and produce industries. In this chapter, food-processing flowcharts are represented from the perspective of food contact materials. Chapter 6 is dedicated to biofilm sampling and detection methods, and Chapter 7 addresses current and future biofilm management control, with a focus on good hygiene practices. The final chapter (Chapter 8) discusses basic and specific hygiene requirements for food production facilities regarding the design, fabrication, and installation of equipment.

This book provides updated information on biofilm growth conditions, detection methods, as well as prevention and control strategies. It offers value as a textbook for students' education about hygiene, but also for hygiene and food technology professionals as a set of guidelines for assuring good sanitation conditions.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Assist. Prof. Dr Mojca Jevšnik, Assist. Prof. Dr Martina Oder, and Dr Sebastjan Filip for their valuable comments and suggestions when reviewing the manuscript, and thanks Terry T. Jackson for grammar proof.

List of Abbreviations

AISI – American iron and steel institute

ATP – Adenosine Tri-Phosphate

a_w – Water activity

CFU – Colony forming units

CIP – Cleaning in place

COP – Cleaning out of place

DLVO - Derjaguin and Landau, Verwey and Overbeek

DNA – Deoxyribonucleic acid

e DNA – Environmental Deoxyribonucleic acid

EDTA - Ethylenediaminetetraacetic acid

EPS – Exopolysaccharide substances

HACCP – Hazard analysis and critical control points

HIV - Human immunodeficiency virus

LED – Light emitting diode

NADH – Nicotinamide adenine dinucleotide

PBS – Phosphate buffer saline

Ra – Arithmetic value of roughness

RLU – Relative light unit

RMAX – MAXIMUM DEPTH OF ROUGHNESS

RQ – ROOT MEAN SQUARE OF ROUGHNESS

RZ – DEPTH OF ROUGHNESS

UV – ULTRAVIOLET

INTRODUCTION

As a result of several bacterial outbreaks in the previous century, biofilms have become a significant public health issue. Consequently, the food industry, public authorities, and consumers are concerned about microbial food safety (Faille et al. 2017b). In the current globally informed world, consumers are becoming increasingly aware of the risks to and consequences for their health. It is assumed that more than 80% of bacterial infections are related to biofilm exposure. In recent years, the globalisation of the food industry has forced food producers to guarantee not only the quality of products but also their safety in response to heightened public awareness. Health issues or even deaths related to the consumption of contaminated food are not just public health concerns but can substantially alter the consumer's trust in a company, damage the company's brand, or influence competitors (Galié et al. 2018).

Biofilm formation is a biological phenomenon as bacteria tend to live on surfaces rather than in a planktonic state; it is very likely that most microbial food contamination is biofilm-related (Brooks and Flint 2008, Azeredo et al. 2017). A solid surface, water, and some nutrients are sufficient to allow the growth of three-dimensional structures. Bacteria can attach to and produce exopolymeric substances (EPS) on various surfaces and food matrixes. Moreover, bacteria can coexist within biofilms with other bacteria or even other organisms, forming so-called multispecies biofilms (Zhao et al. 2017). As a biofilm is formed, its susceptibility to various chemicals and environmental conditions is greatly reduced; therefore, it is difficult to remove it completely. Thus, high doses of biocides cannot remove biofilms as bacteria are well protected by EPS (Zhao et al. 2017, Srey 2013b). The first indications of the problems of biofilms were related to hospital-acquired infections. However, in recent decades, all the materials and surfaces in direct or indirect contact with the human body have come to be considered biofilm exposure risks. In particular, food contact materials are known to be a major factor in the risk of food contamination (Faille et al. 2017b).

Several foodborne illnesses are linked to the presence of biofilms on food contact materials, e.g., *E. coli, L. monocytogenes*, *Salmonella* spp., *B. cereus*, *C. jejuni*, and others (Fink 2015). Therefore, scientists and epidemiological authorities have focused on biofilms when searching for primary sources of food contamination. Not only can biofilms on food contact materials grow pathogenic bacteria, causing health problems for healthy individuals, but facultative pathogenic or even non-pathogenic bacteria in high quantities can have life-threatening impacts on immunocompromised patients.

In industrialised countries, the percentage of people suffering from foodborne diseases each year has been reported to be up to 30% (Bridier et al. 2014, Shapiro et al. 2011). Biofilms can be found on the contact materials of various food industries, including bakery, brewing, seafood processing, ready-to-eat, as well as dairy and meat processing (Fink et al. 2017a). The bacterial contamination of food contact materials is an ongoing concern in the food industry since bacteria can occupy various surfaces and equipment, e.g., conveyors, tables, sinks, benches, floors, and walls. A food contact material's properties are essential for bacterial adhesion and consequent biofilm formation. Surface characteristics (e.g., roughness, surface charge, morphology, and chemical composition) can significantly affect bacterial adhesion. Thus, only by selecting appropriate materials in a food production facility can the bacterial load be significantly reduced. The most common materials in the food-processing industry are stainless steel, polyethene, polypropylene, polyethene terephthalate, rubber, polyurethane, Teflon, wood, and glass (Satpathy et al. 2016).

However, poor sanitation, favourable environmental conditions, and a lack of staff training can also lead to food poisoning (Oder et al. 2015). Furthermore, biofilms cause food spoilage, decrease cleaning efficacy, reduce heat transfer, and even cause obstructions in equipment (Cappitelli, Polo, and Villa 2014). It is reported that thermophilic bacteria may grow on heat exchangers and reduce their effect. This causes problems, especially in the dairy and brewing industries due to foulant build-up in thermal processes (Møretrø and Langsrud 2017).

The food industry provides favourable conditions for biofilm growth due to the prolonged contact time between food and food contact materials, as well as the presence of nutrients, moisture, and bacteria from raw materials (Myszka and Czaczyk 2011). There is no general regulation about what levels of bacteria are acceptable on food contact materials, especially not regarding biofilms. Although most of the methods for biofilm detection are laboratory oriented and not feasible for application, some indirect methods allow the assessment of biofilm biomass, which is more relevant information for food production facilities than the number of cells.

Central to the entire discipline is sanitation as an applied science that includes principles of design, development, implementation, maintenance, restoration, and the improvement of hygienic practices and conditions. The use of good hygiene practice against biofilms in food production facilities can prevent microbial niches and harbourage sites, facilitate cleaning and disinfection, maintain or increase product shelf life, and improve food safety (Marriott 2018). The food industry traditionally uses cost-effective chemical approaches (e.g., sodium hydroxide, ozone, hydrogen peroxide, or sodium hypochlorite) as well as physical methods including high-pressure cleaning, scrubbing, and others. Nonetheless, some of the chemicals cause bacterial cross-resistance, material alteration, and impact the environment and human health (Fink, Kulaš, and Oder 2018).

Novel strategies have been developed in recent years, taking into account the knowledge of the biofilm phenomenon, modern food-processing lines, good hygiene practice, and material science with the aim of reducing the bacterial resistance potential and side effects (Simões, Simões, and Vieira 2010). Effective biofilm sanitation is essential to the attainment of food safety. The continued interest of consumer demand, safe products and security, and high-volume food processing have increased the need for improved good hygiene practice from processing to consumption. By understanding the relationship between food contact materials and bacterial adhesion, strategies can be developed that can help to significantly inhibit, if not prevent, the growth of biofilms.

CHAPTER ONE

PATHOGENIC AND SPOILAGE BACTERIA IN THE FOOD INDUSTRY

Introduction

Foodborne illness is any disease resulting from the spoilage of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food. A foodborne disease outbreak is when two or more persons are experiencing a similar illness, usually gastrointestinal, after eating the same food, if an analysis identifies the source of illness. Pathogenic bacteria can contaminate the food of both animal and non-animal products during primary production, harvest and slaughter, transportation, food processing, storage, distribution, preparation, and serving (Bintsis 2018).

In contrast, food poisoning is an illness caused by the consumption of food containing microbial toxins or chemicals. Therefore, foodborne illnesses caused by a combination of food intoxication and food infections are food toxicoinfections (Marriott, Schilling, and Gravani 2018). Common symptoms are nausea, vomiting, diarrhoea, and abdominal cramps. The process of foodborne illness starts with the source of contamination, followed by the transfer of bacteria, potentially risky food preparation, consumption, and food poisoning. The chain of infection demonstrates a series of related events of factors that exist and have to be linked together before infection will occur (CDC 2012). From the food contact material point of view, surfaces in food facilities can represent the reservoir of microorganisms or mode of transmission. A reservoir is defined as a location where pathogenic microorganisms live, grow, and multiply. Food contact materials are ideal environments for microorganism reservoirs. Moreover, cross-contamination by food contact materials represents an important indirect vehicle of microorganism transportation (Fig. 1.1) (CDC 2012).

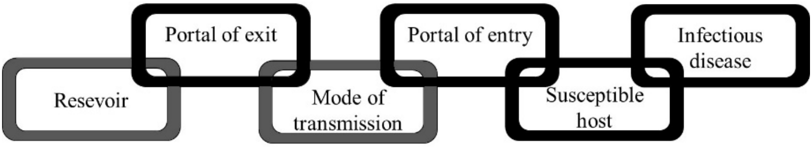

Figure 1.1. The chain of infection from a food contact point of view (CDC 2012)

Although anyone can contract a foodborne illness, especially at risk are pregnant women, young children, older adults and people with weakened immune systems and chronic illnesses. The first estimates of the global burden of foodborne diseases show that almost 1 in 10 people fall ill every year from eating contaminated food, and 420,000 people die as a result. The World Health Organization extensively investigated the global burden of foodborne disease and stated that the problem is comparable to major infectious diseases, such as HIV, malaria, and tuberculosis. The report pointed out the the most frequent cases of foodborne diseases are diarrheal disease agents, particularly *Campylobacter* spp. and *Salmonella enterica*. The estimated disability-adjusted life years due to foodborne diseases worldwide are 33 million years; children under five years bear almost half of this burden (Havelaar et al. 2015).

Cross-contamination is the most significant vehicle of transmitting bacteria to food. In the food industry, cross-contamination is defined as direct or indirect bacterial transference from a contaminated to a non-contaminated matrix by food, workers, or food contact materials. In contrast to that, recontamination is the contamination of food after it has undergone a sanitising treatment (González-Rivas et al. 2018).

Dominant bacteria that form biofilms in the food industry

Bacteria are unicellular, simple structures, rod-like, spherical, or curved, ranging in size from 0.1 to 10 microns. They constitute a large majority of prokaryotic microorganisms. The largest number exist in the gut flora, and many also live on the skin. The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and many are beneficial, particularly the gut flora. However, several species of bacteria are pathogenic and cause infectious diseases (Talaro and Chess 2018).

Due to the simplicity of bacterial genetic material, their cells can occupy various environments among food contact materials; this is significant from a hygiene point of view (Sharif et al. 2018).

The contamination of food may occur at any stage in the process from food production to consumption and can result from environmental contamination, including the pollution of water, soil, or air (Fink 2015, Simões, Simões, and Vieira 2010). It is important to highlight the fact that the growing, harvesting, preparing, storing, processing, and distributing of food represent risks for microbial contamination (Sharif et al. 2018).

Industrial surfaces can provide a solid substrate for biofilm formation. *Bacillus cereus*, *Escherichia coli*, and *Salmonella* spp. have been found in meat, dairy, and egg-processing facilities; *Listeria monocytogenes* and *Staphylococcus aureus* have been detected on industrial equipment surfaces in seafood production facilities (Bridier et al. 2015). The most common symptoms of foodborne diseases are gastrointestinal symptoms, but such diseases can also have neurological, gynaecological, immunological, and other impacts. In addition, multi-organ failure and even cancer may result from foodborne diseases, thus representing a considerable burden of disability as well as mortality (Talaro and Chess 2018).

Several public health authorities have linked foodborne illnesses with exposure to biofilms. Recent studies on foodborne outbreaks have focused on biofilms, examining the sources of food contamination. Bacterial biofilms can grow on food matrixes or food contact materials and cause health issues (Camargo et al. 2017, Galié et al. 2018).

Bacillus cereus

B. cereus is a rod-shaped, anaerobic or facultatively anaerobic Gram-positive and spore-forming bacterium that can grow in different environments and a wide range of temperatures (Chaves et al. 2017). The natural habitat of this bacterium is dust, water, and soil. Therefore, it is present in many foods and food ingredients. *B. cereus* can proliferate in a pH range between 5 and 9 and minimum water activity of 0.98 (Rao et al. 2018). Cells of *B. cereus* are large and motile; it has been shown that motility plays a significant role in bacterial adhesion and biofilm formation (Wirtanen and Salo 2016).

B. cereus is often found to be resistant to heat, chemical treatment, and radiation (Bottone 2010). Moreover, these cells can survive high temperatures (e.g., industrial pasteurisation) due to the production of endospores. It is well described in the literature that spores are much harder to eliminate from surfaces than vegetative forms are, which impairs cleaning procedures (Auger et al. 2009; Fink et al. 2017b; Gopal et al. 2015) and increases the risk of outbreaks.

B. cereus is a typical representative of a bacterium that causes food poisoning. Some of the strains can produce diarrheal enterotoxins, which cause abdominal pain and diarrhoea, while other strains produce emetic toxins, which cause vomiting symptoms. From a public health perspective, the production of bacterial haemolysins is of particular importance since exposure can result in fatal dehydration or even death (Tschiedel et al. 2015). Diarrhoea from *B. cereus* is associated with the consumption of a diversity of food types. In contrast, *B. cereus* emetic syndrome is caused by cereulide, which is related to the toxic activity of this heat-stable peptide to the mitochondria. The emetic type is associated with rice and pasta consumption. It is reported that an infective dose for a human is between 10^5 and 10^8 CFU of *B. cereus* (Galié et al. 2018; Soni et al. 2016). Commonly known *B. cereus* foodborne outbreaks are related to rice consumption, re-warmed mashed potatoes, cereals, puddings, milk products, soups, and meat (Chaves et al. 2017).

B. cereus tends to form biofilms on various types of food contact materials. Moreover, *B. cereus* biofilms are often associated with other bacteria in food processing due to exopolysaccharides, proteins, and extracellular DNA, which are necessary for adhesion (Majed et al. 2016). On surfaces, these substances have a preconditioning effect since they facilitate the fast attachment of other bacterial species that would otherwise be removed by water flow, milk streams, or other physical mechanisms present in these industries (Marchand et al. 2012).

Biofilms that consist of *B. cereus* cells are commonly found in the dairy industry, bakeries, and beverage production facilities (Ehling-Schulz, Frenzel, and Gohar 2015; Fink, Oder, et al. 2017). In these facilities, *B. cereus* biofilms are found in both air-liquid and submerged surfaces, such as in stainless steel tanks and pipes (Hayrapetyan et al. 2015). The authors also pointed out that *B. cereus* tends to attach to stainless steel more firmly than to polystyrene due to the availability of iron in stainless steel surfaces. It was reported that enterotoxin in milk could occur in 7-8 days, and that milk products with high butterfat are riskier, as are products processed with high-temperature and short pasteurisation treatments (Saleh-Lakha et al. 2017). A study in Brazil revealed that *B. cereus* was present in one third of the entire dairy industry (Chaves et al. 2017), which represents a major public health issue, especially with regard to the exposure of at-risk groups.

Escherichia coli

E. coli is a Gram-negative, optional anaerobic, rod-shaped, coliform bacterium that is commonly found in the lower intestines of warm-blooded organisms. The bacterial cells can start to grow at 8 °C with the optimum in a range 30-42 °C. The optimal pH environment is between 5.5 and 7.5, although several strains can grow in much lower pH environments (Aquino et al. 2017). The presence of intestinal cells of *E. coli* in water and food indicates a potential hygiene hazard.

Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts and are responsible for product recalls due to food contamination. *E. coli* often contaminates tomatoes, melons, lettuce, milk, meat, and other foodstuffs. Generally, foodstuff is contaminated during the pre-harvest period due to contaminated water irrigation. Furthermore, post-harvest *E. coli* contamination can occur during the washing and the cleaning of the surfaces of vegetables and fruits, in storage temperatures that allow the growth of colonised bacteria, and with foodstuff handling (e.g., slicing, peeling, and cutting) (Carter et al. 2016; Galié et al. 2018).

Bacterial cells of *E. coli* tend to attach themselves to different food contact materials commonly found in food industry and kitchen environments. The common materials that are usually colonised with *E. coli* are stainless steel, Teflon, glass, polystyrene, polypropylene, PVC, and biotic surfaces (e.g., food surfaces, skin, hair) (Carter et al. 2012; Bohinc et al. 2015). For example, initial attachment of *E. coli* and consequently biofilm development on the surfaces is enhanced by the presence of flagella and fimbria (Van Houdt and Michiels 2010). *E. coli* resistance to environmental stress conditions and biofilm formation strongly depends on the strain. Environmental stress can trigger the expression of genes, which will result in flagella and fimbria development. This will allow bacteria to attach to the surface more firmly than they would in normal conditions. *E. coli* O157:H7 causes haemorrhagic colitis and haemolytic uremic syndrome and represents a great threat to the food industry. Moreover, it was found that *E. coli* O157:H7 is strongly resistant to temperature, pressure, cleaning agents, and disinfectants in comparison to other pathogenic strains (Chagnot et al. 2014).

Some studies reported that *E. coli* are strongly resistant to acidic environments that allow cells to survive in extreme conditions, including the production of fruit juice, and fermented dairy, vegetable, and meat products (Lajhar, Brownlie, and Barlow 2017). For example, acid-adapted *E. coli* has been found to form strong biofilms on stainless steel surfaces

(Lelieveld, Holah, and Gabric 2016). This fact is of significant importance with respect to health issues, as surviving enterohemorrhagic *E. coli* O157:H- and O145 cells stored in acidic food matrixes were more resistant to gastric acid challenge and, therefore, a lower infective dose is needed (McLeod et al. 2016).

Another health issue is Shiga toxin-producing *E. coli* that cause enterohemorrhagic gastroenteritis with watery diarrhoea and blood in the faeces. The risky foods for intoxication are soups, sauces, cooked chicken, ground beef, salads and other fresh products; food contaminated with this strain has led to outbreaks and even death (Yang et al. 2017).

The infectious dose for *E. coli* O157:H7, is less than 50 CFUs. Therefore, even low-grade biofilm contamination of a food facility is a serious health problem and requires strong control measures (Marouani-Gadri et al. 2010).

Listeria monocytogenes

The Gram-positive bacteria *L. monocytogenes* is a ubiquitous, facultative, anaerobic, non-spore forming, opportunistic pathogenic bacterium. Natural reservoirs are humans, birds, sheep, cattle, chickens, and pigs. Other potential sources are stream water, sewage, mud, trout, flies, crustaceans, and ticks (Colagiorgi et al. 2017).

Cells can grow and reproduce inside the host cells; it is one of the most virulent foodborne pathogens; 20 to 30% of foodborne listeriosis infections in high-risk individuals may be fatal. It can cause miscarriages in pregnant women and be fatal in immunocompromised individuals and the elderly. In healthy people, with strong immune systems, listeriosis generally only causes a mild form of illness. The temperature growth range of *L. monocytogenes* is between -1.5 and 45 °C, therefore it is not resistant to pasteurisation (Milillo et al. 2012). *L. monocytogenes* is problematic for the seafood, meat, dairy, ready-to-eat, frozen vegetable, and poultry food industry sectors (Rothrock et al. 2017). The cells can persist in food-processing plants for several years, which can be a significant factor of biofilm spreading and the reason behind a number of outbreaks (Ferreira et al. 2014). Commonly contaminated foods include milk products, meat, vegetables, smoked fish, and fresh cheese.

L. monocytogenes biofilms can occupy various materials used in the food industry, e.g., polypropylene, steel, rubber, or glass. It has been found in all parts of pork slaughtering and cutting plants; cells were also present in lairage pens from which bacteria are spread in the environment (Larivière-Gauthier et al. 2014). More significantly, *L. monocytogenes* can replicate at refrigeration temperatures (Silva et al. 2008). Moreover, biofilm production of *L. monocytogenes* is induced by low temperatures and cell hydrophobicity by increasing the ability to attach to the surface and the production of EPS. Eradication of such biofilms is complicated due to its resistance to cleaning agents and temperatures below 60 °C (Galié et al. 2018). It has been reported that *L. monocytogenes* can form multispecies biofilms with other meat and lactic acid bacteria (e.g., *Pseudomonas* spp., *Enterobacteriaceae*) with the potential to increase resistance to chemicals (Puga, Orgaz, and SanJose 2016).

Salmonella

Salmonella spp. is a rod-shaped, flagellate, facultatively anaerobic, Gram-negative bacterium, and a species of the genus *Salmonella*. It is one of the most significant pathogens transmitted via food, especially by poultry, and can form biofilms on various materials (Ben Abdallah et al. 2014).

Salmonella spp. have adapted to a wide range of environmental conditions, such as variation in temperatures, pH, desiccation, available nutrients, antimicrobial substances, and even food preservatives (Lamas et al. 2018).

The ability to grow biofilms is related to gene expression, cell surface characteristics, and environmental conditions. It has been reported that biofilm formation is strongly promoted on stainless steel surfaces when food contaminants (e.g., chicken exudate and milk) are added (Lamas, Regal, et al. 2018). Moreover, *Salmonella* uses aggregative fimbriae to attach to surfaces and can produce large amounts of cellulose to build EPS (Giaouris, 2018). Another key factor that promotes biofilm is quorum sensing, which works through the production of small molecules' autoinducers and their gene-encoded receptors (Lamas, Paz-Mendez, et al. 2018). Scientists have demonstrated that *S. enterica* on hydrophobic and low roughness surfaces attach in lower amount in comparison to material that is more hydrophilic and rougher (Dhowlaghar et al. 2018).

This bacterium causes nausea, vomiting, fever, diarrhoea, and abdominal pain as the main symptoms (Nguyen, Yang, and Yuk 2014). The infectious dose can be as low as one to ten cells. It can grow in temperatures between 5 to 50 °C, pH 6.5–9.5 and water activity of 0.86. *S. enterica* is commonly found in poultry food-processing facilities, causing biofilm formation on stainless steel (Wang et al. 2013). Moreover, it has been demonstrated that *S. enterica* can increase resistance to heat in biofilm form in the presence of wheat flour in comparison to a single cell.

This strongly reinforces the necessity of biofilm removal in food-processing facilities even with low-moisture food (Villa-Rojas et al. 2017). The study that simulated an egg-processing environment revealed that most *E. enteritidis* strains form biofilms on stainless steel surfaces. Moreover, the research found that biofilms grown in egg media are far more resistant to chlorine and hot water treatment than biofilms grown in laboratory media (Yang et al. 2016).

Staphylococcus aureus

S. aureus is a Gram-positive, non-spore-forming, non-motile, facultatively anaerobic bacterium. It is an opportunistic pathogen, largely due to its characteristic production of enterotoxins at temperatures between 10 and 46 °C. The cells are very adaptable and can live in a wide variety of environments as biofilms. Furthermore, biofilm formation is considered to be a significant virulence factor for bacteria of the genus *Staphylococcus* (Di Ciccio et al. 2015). Humans are common asymptomatic carriers of *S. aureus* in their noses, throats, and skin. *S. aureus* can multiply on the mucous membranes and skin of food handlers, can be spread by cross-contamination during food preparation and, therefore, represents a significant risk to the food industry (Giaouris et al. 2015). Commonly known foodstuffs involved in staphylococcal poisoning include meat, poultry, eggs, dairy and milk products, as well as cream-filled bakery products (Silva et al. 2017).

S. aureus enterotoxins are heat-stable and are secreted during growth into a food matrix. Moreover, even foods with low water activity (high sugar or salt content), which are generally recognised as safe, are good media for *S. aureus* growth (Schelin, Susilo, and Johler 2017; Galié et al. 2018). A study in meat-processing facilities demonstrated the resistance of *S. aureus* to a 3% solution of peracetic acid and 25% sodium hypochlorite (de Souza et al. 2014). *S. aureus* biofilm formation can be triggered by suboptimal growth temperatures, and the presence of nitrite, sodium chloride, and even the presence of alcohol can induce haemolytic properties of *S. aureus* biofilm. One study demonstrated that *S. aureus* can form intensive biofilms in environmental conditions, e.g., 3.5% ethanol concentration, neutral pH and a_w of 0.98 (Tango et al. 2018).

Moreover, it was observed that the presence of natural microbiota from raw food can perform conditioning, favouring the anchoring of *S. aureus* to the surface (Marchand et al. 2012). The factors that promote *S. aureus* biofilm growth are suboptimal temperatures, improper disinfection, or a combination of salt and glucose. Using sub-lethal concentrations of various common detergents used in the food industry causes the transcription of genes involved in biofilm formation, toxin secretion, and adhesion (Vergara et al. 2017; Slany, Oppelt, and Cincarova 2017).

Campylobacter jejuni

Campylobacter jejuni is a non-spore forming, Gram-negative, curved or spiral-shaped rod. The cells grow intensively in a microaerophilic environment; therefore, increasing levels of CO_2 (up to 10%) will promote biofilm formation (Percival et al. 2011). This pathogen is the most frequent cause of bacterial gastrointestinal foodborne infection worldwide. In addition to foodborne pathogens, oxidation is a non-microbial cause of the deterioration of food, causing loss of quality and safety. According to the European Food Safety Authority, campylobacteriosis is the most persistent infectious disease related to food consumption. More than 245,000 cases or 66 cases per 100,000 people were recorded in 2016 (EFSA 2017). Although the number of campylobacteriosis infections is high, their severity regarding reported case fatality is low (0.03%) (García-Sánchez, Melero, and Rovira 2018). Nonetheless, campylobacteriosis is the third most common cause of mortality among major pathogens (EFSA 2017).

The bacteria are transmitted via contaminated food, most often with poultry. The cells have been found on fresh and frozen retail poultry products; moreover, it has been shown that the intestinal tract of poultry is a good reservoir for *Campylobacter*, containing up to 10^8 CFU/g (Teh, Flint, and French 2010). Several outbreaks have been related to the consumption of unpasteurised cow milk, unripe cheese products, and unpasteurised sheep's milk (Koppenaal et al. 2017).

Campylobacter biofilms have been found in the watering supplies and plumbing systems of animal farm facilities and processing plants. In the poultry industry, interactions between different strains of *C. jejuni* may have implications for cell survival on surfaces (Teh, Flint, and French 2010). *Campylobacter* forms biofilms on food contact surfaces, including stainless steel and polystyrene plastic. On such smooth surfaces, flagella-mediated motility has been found to be a significant activity in biofilm formation (Percival et al. 2014).

When compared to other foodborne pathogens, *C. jejuni* seems to be the most susceptible to stressors; therefore, it is assumed that biofilm formation plays an important role in cell survival (Bronowski, James, and Winstanley 2014). Indeed, resistance to antimicrobial agents, detergents, and disinfectants might be increased by biofilms. It has been reported that exudate can have a significant contribution to biofilm formation in poultry-processing facilities by providing nutrients and conditioning film on abiotic surfaces (Brown, Hanman, et al. 2015). It has been shown that the incidence of campylobacteriosis has clear seasonality, with sharp peaks in the summer months (EFSA 2017). Moreover, it seems that Africa, Asia, and the Middle East are endemic regions for Campylobacter infections (García-Sánchez, Melero, and Rovira 2018).

Conclusions

Pathogenic bacteria usually do not change the taste, colour or texture of food, so it is difficult to recognise contamination. Usually, efforts are not made to remove bacterial cells from surfaces unless overgrowth or visible spoilage occurs. Food infection or intoxication can appear even when food is contaminated in low quantities of pathogenic bacterial cells (Akyar and Picó 2015). It is known that biofilms in food facilities can secrete toxins and cause contamination of the food matrix, resulting in individual or multiple intoxications. The risk of foodborne disease depends mainly on bacterial species and its infective dose, causing illness. The most dominant vehicles for bacterial cross-contamination are raw materials, food debris, and surfaces such as pipelines, pasteuriser plates, tables, and contact surfaces, as well as employees (Camargo et al. 2017; Galié et al. 2018).

Biofilm growing on food contact materials cause several health and processing problems, as these structures are hard to remove. Moreover, bacteria are becoming increasingly resistant to known chemicals and, therefore, forcing the food industry, researchers, and public health authorities to find new strategies to combat emerging food pathogens. The optimal biofilm management for food production facilities requires knowledge of pathogens, their special growth conditions, and factors affecting cell adhesion and the formation of biofilms.

CHAPTER TWO

FOOD CONTACT MATERIALS

Introduction

Before it is consumed, food comes in contact with many surfaces during its production, processing, storage, preparation, and serving. Food contact materials are either intended to be brought into contact with food, are already in contact with food, or can reasonably be brought into contact with food or transfer their constituents to the food under normal or foreseeable use (Cruz Garcia et al. 2014).

Food contact materials are included in all stages of food production and are used as containers for transporting, machinery for processing, packaging and kitchenware and tableware. However, every different player in the food chain seems to consider food contact materials in a different way. They are seen by inexperienced subjects with reference to the real edible content. However, modern food packaging is considered an essential basis of food safety strategy worldwide (Barone et al. 2015). It is made from a variety of materials, including plastics, wood, glass, rubber, paper, and metal. Materials used in processing equipment of the food industry and containers used for transport are also considered (Fink 2015).

The choice of material when designing food-processing facilities is a vital step in providing safe and high-quality products. Materials used in the food industry must meet the requirements regarding the low potential of microorganism adhesion, corrosion resistance, mechanical strength, longevity, nontoxicity, weldability, and ease of fabrication (Kanematsu and Barry 2015a).

Contemporary food production represents ideal conditions for biofilm formation on food contact materials due to the complexity of processing equipment, the industrial scale of production, the length of production processes and the vast surface areas available for biofilm development (Lindsay and Holy 2006). Food contact materials (e.g., cutting tools, food crates, and conveyor belts) are often contaminated with pathogenic bacteria due to frequent use and long contact times with food products (Giaouris and Simões 2018b; Fink, Oder, et al. 2017). Generally, in food production, daily sanitation plans enable removing the bacteria from surfaces with cleaning and disinfection approaches. However, so-called ‘dead zones’ (e.g., drains, walls and ceilings, pipelines, pumps, valves, gaskets, corners, and joints) represent risks for microbial contamination. Such surfaces may not receive sufficient exposure to cleaning and disinfection agents. Cracks, joints, and crevices can provide hiding sites for bacteria. Due to the relatively larger surfaces and roughness of such surfaces, bacterial cells form biofilm communities, which may prevent them from being removed by chemicals (Liu et al. 2016; Giaouris and Simões 2018a).

Depending on their stability, materials intended for contact with food are divided into two groups: those intended for single use (disposable) and those intended for reusable use.

Disposable materials

Disposable materials are materials that the user will use once, and they are expected to be discarded after use. Typical disposable materials include paper and cardboard, wood, and polymer materials (e.g., cardboard boxes for pizza, plastic bags for frozen, cold, and dry storage foodstuffs, plastic skewers, bags and foils for foodstuffs, etc.) and other materials intended to protect foods in terms of the primary packaging of various foods (e.g., Tetra Pak packaging for juices and milk, cheese wax, etc.). Disposable food packaging comprises disposable items often found in fast food restaurants, takeout restaurants and kiosks, and catering establishments. By being used only once, these products significantly reduce food contamination and the spread of diseases (Fink et al. 2017a).

Reusable materials

Reusable materials are manufactured from durable materials and are specifically designed for multiple uses and extended life. Reusable materials are designed for reuse without impairment of their protective function. Common representatives of this group of food contact materials are glass, ceramics, alloys, silicones, polymeric materials, rubber, textiles, wood, and other materials. The application includes all cooking utensils, a programme of cooking and serving dishes, containers intended for storing foodstuffs, transport containers, preparation devices (e.g., potato peelers, shockers, cutters, mixers, etc.), thermal food processing (e.g., grills, fryers, toasters, convectomats, ovens, etc.), storage of foodstuffs (e.g., tanks, etc.), production lines (e.g., production lines for juices, pasta, bread, etc.),