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Zahra H. Mohammad
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Sadaf Zaidi *Editors*

Microbial Biotechnology in the Food Industry

Advances, Challenges, and Potential Solutions

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Dedicated to family and friends of all editors
for their support and encouragement

Preface

Biotechnology is a promising and emerging technology that uses biological systems to develop new products. Biotechnology has a broad application in different areas, including medical biotechnology, industrial biotechnology, environmental biotechnology, and marine biotechnology. Biotechnology in the food industry could provide solutions to microbial issues and the food industry environment.

Biotechnology has a significant role in sustainable agriculture, food processing, and food preservation sectors, including the development of transgenic enzymes, fermentation, increasing crop varieties that are resistant to disease and pests, increasing yield, increasing the production of poultry and cattle, modification of microorganisms, inhibition of diseases and pathogen-causing diseases, protection of the environment, and much more through gene modification. The application of modern biotechnology also improves the taste, nutrition, and shelf life of foods. Hence, biotechnology has a vital role in the food sector and has great potential to resolve the future starvation risk with the growing population.

This book considers how microbial biotechnology plays an important role in different areas of the food industry like microbial biofilms and the role of biotechnology as a solution, the application of microbial enzymes in the food industry, roles of biotechnology in environmental monitoring, biotechnology and its position in the mitigation of microbial problems in the food industry, and use of microbe-free contact surfaces to control food spoilage. Mainly, the book focuses on microbial issues in the food industry and the solutions using novel biotechnology techniques. Moreover, it comprehensively provides an informative state-of-the-art perspective of microbial biotechnology in the food industry, focuses on biotechnology approaches for food security, risks, and solutions, discusses food laws and regulations related to food security and microbial contamination in the food processing environment, and presents bioprocesses and biosystems for environmental protection, microbial detection, and prevention in the food industry and wide range of topics related to specific solutions using biotechnology from eminent experts around the world.

However, the book contains a total of 19 chapters on different aspects of the application of microbial biotechnology. The first chapter overviews different biotechnology approaches for food security, risks, and possible solutions to food problems. Other chapters discuss microbial contamination in the food processing environment, the risk of contamination, the consequences of microbial contamination, and their solutions. Some chapters also highlight the use of microbe-free contact surfaces to control food spoilage. Microbial biofilms on food contact surfaces and the role of biotechnology are also discussed. The book mainly highlights the applications of microbial biotechnology in the food industry. One of the chapters discusses food laws and regulations related to food security. The application of nanoparticles to enhance food products' microbial quality and shelf life is also mentioned. Some chapters discuss the roles of biotechnology in environmental monitoring in the food industry and the use of bioprocesses and biosystems for environmental protection, microbial detection, and prevention in the food industry. Reports on the application of biotechnology and microbial enzymes in the food industry are also summarized. Effects of water contamination on food safety and related health risks, issues, and perspectives of film-based packaging for food safety and preservation are also discussed. Chapters also highlight the challenges in environmental biotechnology and the role of air and aerosols in contaminating food products during food processing. One of the approaches of healthcare management – probiotics and its delivery systems – are also discussed.

We thank all contributing authors who have generously given their time and expertise in preparing very informative chapters on the subject. We are also highly thankful to all the reviewers for their valuable time and contribution to the peer-review process. All the technical support and assistance that Springer provided during the book's preparation and production is gratefully acknowledged. Lastly, we thank our family members for their love, support, encouragement, and patience during the entire period of this work.

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Faizan Ahmad, M.Tech., Ph.D., is currently working as an assistant professor at the Department of Post Harvest Engineering and Technology, Faculty of Agricultural Sciences, Aligarh Muslim University, India. Dr. Ahmad has completed his B.Tech. and M.Tech. degrees in Chemical Engineering from Zakir Husain College of Engineering and Technology, Aligarh Muslim University, and Ph.D. in Post Harvest Engineering and Technology from the Department of Post Harvest Engineering and Technology, AMU, Aligarh, in post-harvest quality assessment of fruits and vegetables. He has more than 10 years of teaching and research experience. His current research is focused on developing food packaging by incorporating essential oils and nanoparticles. His research also includes quality evaluation of fruits and vegetables, food processing, preservation, and new product development by incorporating agricultural waste. He has published around 20 research papers in international and national reputed journals and more than 15 book chapters. He has edited one book, *Quality Control in Fruits and Vegetable Processing: Methods and Strategies*, published by Apple Academic Press (C.R.C. Press, Taylor & Francis Group). He has also supervised about 12 M.Tech. dissertations and currently supervises 1 Ph.D. and 4 M.Tech students. He is a life member of the Indian Society of Technical Education (ISTE). He also has one patent granted by Indian Government.

Zahra H. Mohammad, Ph.D., is currently working at Advanced Health and Education Services Organization as CEO/president of the organization, managing various training and research programs. She previously worked as a Food Microbiology and Extension Specialist at the University of Houston for many years. She has completed a B.Sc. degree in Chemical Engineering. She was awarded a master's and Ph.D. in Food Science and Technology/Food Microbiology from Texas A&M University. Her research interests focus on detecting pathogens in food, water, and food-related environments and how to counteract, prevent, and control their potential threat. She took a leading role in a wide variety of research projects addressing food safety, the environment, and the quality and shelf life of foods using conventional and modern intervention technologies, including thermal, non-thermal, molecular, and nanotechnology approaches. She has more than 10 years of teaching, extension, and industry experience and has published more than 30 publications in peer-reviewed journals.

Salam A. Ibrahim is a Professor of Food Science in the Food and Nutritional Science Program at North Carolina Agricultural and Technical State University, USA. He has established a research program around dairy technology, yogurt starter cultures, probiotics, bioconversion processes, food safety, and the use of natural compounds to inhibit the growth of foodborne illnesses. His program is well-funded by the National Institute of Food and Agriculture-United States Department of Agriculture (NIFA-USDA), the Department of Homeland Security (DHS), and other funding agencies. In addition, the food microbiology laboratory has strong connections with the food industry and often receives funds to support students around fermentation and the production of stable and functional probiotics products. Dr. Ibrahim is specifically interested in *Lactobacillus bulgaricus*, recognized in the early 1900s as a health-promoting starter culture in yogurt. Currently, Dr. Ibrahim is working on novel methods to produce stable and functional lactic acid cultures for the dairy industry.

Sadaf Zaidi M.Tech., Ph.D., is currently working as a professor at the Department of Post Harvest Engineering and Technology, Faculty of Agricultural Sciences, Aligarh Muslim University, India. Prof. Zaidi completed his Bachelor of Science degree and the M. Tech. degree in Chemical Engineering from Aligarh Muslim University, Aligarh, India. He was awarded the Ph.D. degree in Chemical Engineering from the same university. His teaching and research experience spans about 34 years. Besides teaching several UG and PG courses, he has successfully supervised 6 Ph.D. and 22 M.Tech. students. At present, he is guiding 5 Ph.D. and 5 M.Tech. students. His research interests include artificial intelligence, processing and food engineering, renewable energy, waste management, modelling, and simulation. Prof. Zaidi has published more than 50 research papers in reputed SCI journals. He has co-edited one book on electrochemical capacitors. He has contributed seven chapters in knowledge-based editions published by reputed publishers like Nova Science Publishers Inc. (USA), Springer Science and Business Media BV, Springer Singapore, Intech Open (UK), Materials Research Forum LLC (USA), etc. He has published 15 research papers in conference proceedings and contributed/presented more than 60 working papers at national and international conferences and symposia. He has also served the Department of Post Harvest Engineering and Technology, AMU, Aligarh, as its chairperson from 2020 to 2023. He is a life member of the Indian Institute of Chemical Engineers (IIChE).

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Chapter 1
Biotechnology Approaches to Food Security: Risks and Solutions

Zahra H. Mohammad, Faizan Ahmad, and Salam A. Ibrahim

Introduction

Food security, agricultural sustainability, and hunger issues in developing countries continue to be a worldwide concern (Challa et al., 2019; Mustafa, 2020). Food security is a subject of great concern due to the growing population, urbanization, and the consequences of increasing demands on food supplies (Beddington, 2010). The general definition of food security is the ability to secure a healthy food supply to feed the whole population, maintain a healthy life, and be available at any time (Challa et al., 2019). Based on the Food and Agriculture Organization of the United Nations (FAO), food is secure when it is available, able to be accessed, and suitable for consumption. Due to global climate changes and a fast-growing population, the concept of food security has changed over the last 55 years (Challa et al., 2019) (Fig. 1.1).

Today, 800 million people worldwide are suffering from hunger, and the population continues to increase every day. The global population is estimated to increase to 8.3 billion by 2030 (Baulcombe, 2010). It is estimated that global food production must increase by 50–100% in order to meet food availability (United Nations, 2017). Thus, food production must dramatically increase in order to address and overcome starvation challenges (Godfray & Garnett, 2014).

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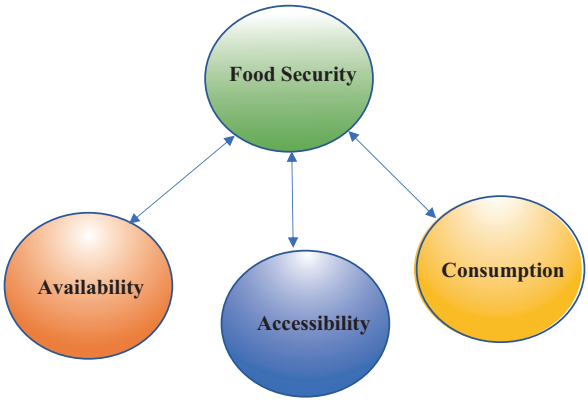

Fig. 1.1 The success of food security

Climate change has affected the earth's water sources, land, and soil, and we are losing clean, safe drinking water on a regular basis. The critical solutions are agriculture, increasing productivity improvement of plants, and introducing novel approaches to food processing (Ghoshal, 2018). Therefore, innovative technologies that ensure sustainable agriculture and improve plant productivity are paramount. Biotechnology approaches must be given more attention and take advantage of their innovative applications to ensure food security and an adequate food supply both now and in the future. For example, expanding the application of biotechniques in the agriculture system to include more genetically engineered crops, more focus on bioengineered approaches to improve soil functionality, hybridization, agricultural plant breeding, and other selection activities for agriculture, and increasing the biotechnology applications in the food industry.

Biotechnology is the approach that manipulates living organisms or their materials to convert certain products, enhance plant and animal production, and generate microorganisms for specific purposes (Abah et al., 2010). The innovative biotechnology technique has been used to develop and domesticate plants into tastier, safer, more nutritious, and healthier crops (Pal et al., 2017). Biotechnology is not a new approach, this approach has been in place since ancient times (Copeland, 2017). For example, humans began the process of involving the use of microorganisms in food to produce bread, wine, cheese, and dairy products preservation (Copeland, 2017).

GMOs are examples of a modern biotechnology approach used to modify a plant's DNA in order to enhance the plant's yield, taste, and resistance to diseases (De Souza & Bonciu, 2022). Manipulating DNA and genetic material helps to reduce diseases and adverse environmental effects (De Souza & Bonciu, 2022). As a result, biotechnology can contribute to food security through plant preservation, sustainable agriculture, and food safety. This chapter covers the role of biotechnology in food security, the challenges of using biotechnology to enhance food security, and the risks of using modern biotechnology approaches.

Role of Biotechnology in food security

Biotechnology plays a significant role in food security because of its innovative technology that can be manipulated in agriculture and food processing systems. This provides opportunities to increase food production and, at the same time, control the current challenges, including diseases, climate changes, and so on (Ghoshal, 2018).

Biotechnology is a promising approach for agriculture production and food supplies because it depends on plant genes to control food production (Mustafa, 2020; Scarpato & Ardeleanu, 2014). In this regard, food processing operations took advantage of biotechnology applications and were among the first investors that intensively utilized biotechnology applications earlier and recently (Johnson, 2018; Mustafa, 2020; Scarpato & Ardeleanu, 2014). The applications of biotechnology are currently expanding to involve more in the food chain system (Mustafa, 2020). These allow modern biotechnology to play a significant role in food processing, agricultural sustainability, and future food security by manipulating the agricultural production and food system (Sengar et al., 2016).

Modern biotechnology emerged in the last three decades and brought the very first evolution of gene recombinant. Hence, it changes the way humans live by impacting all aspects of life, including food, health, drink, and other daily needs (Johnson, 2018). The application of modern biotechnology has expanded and positively affected the agriculture and food industries. Biotechnology has great potential to resolve the issue of hunger now and meet the food requirements to prevent starvation in the future. (Ghoshal**,** 2018**).**

Biotechnology contributes to improving various areas, including plant preservation, sustainable agriculture, and food safety (Ghoshal, 2018; Sengar et al., 2016). These three areas can be expanded into sub-aspects, including the production of disease-resistant plants, fortification of crops, increase in aquaculture, pest-resistant crops, drought-tolerance crops, biofuel, enzymes, progress in fermentation, improve plant yield, increase shelf life, improve animal feed (Ranjha et al., 2022) (Fig. 1.2).

Plant Preservation

Since plants are essential sources of life for humans and animals, improving and enhancing the plants must be the primary goal. Thus, securing the required foods for the future growing population and feed for animals (Francis et al., 2017). Biotechnology can do a lot to improve plants and has the ultimate capacity to secure this vital source of humans' lives.

Fermentation is an example of the application of biotechnology in plant preservation that has been dramatically improved (Johnson, 2018). Fermentation is utilized to provide favorable changes to food using microorganisms, such as bacteria or yeast. This process can occur naturally in some foods, but most of the time, the

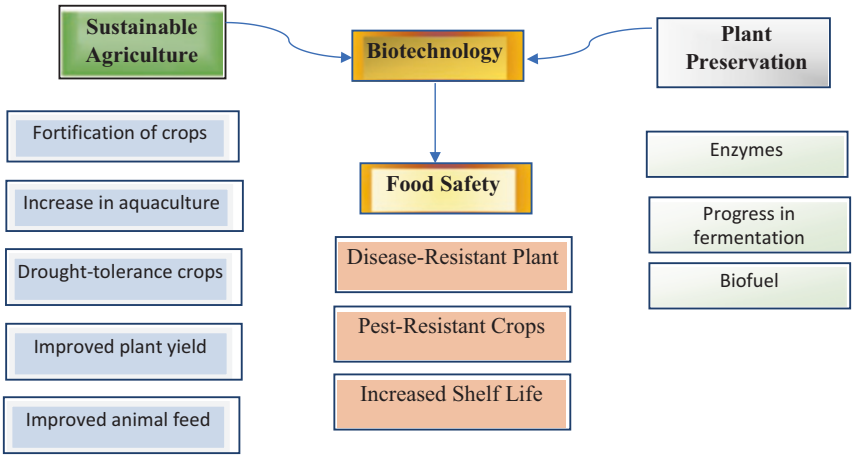

Fig. 1.2 The types of biotechnology

process is achieved by intentionally adding certain bacteria, yeast, or a combination of both under an anaerobic environment (Johnson, 2018). This process is essential in producing beer, wine, lactic acid, vinegar, and bread leavening. Fermentation improves food processes in several areas, including enriching foods with proteins, essential amino acids, and vitamins, preserving foods through acid production, eliminating antinutritional factors, changing diets by modifying flavors, aromas, and texture, and reducing the processing time. The production of these vitamins, amino acids, and other acids has been produced using genetically modified microorganisms (Johnson, 2018). For example, biotechnology can be used to increase the vitamin content in specific crops by transgenic plants.

Recently, with the emergence of modern biotechnology, a new recombinant genetic engineering technique has significantly impacted food fermentation by altering the purified microbial strain related to food fermentations. Recombinant basically means building or generating new microorganisms, animals, plants, or even cells with more valuable features by recombinant DNA methods (Abah et al., 2010).

Another area that biotechnology plays a significant role in is generating various types of enzymes. Enzymes are used by the food industry for food production and food processing (Lokko et al., 2018). These enzymes are generated by using genetically modified microorganisms (Ranjha et al., 2022). Generally, genetically modified applications can be utilized in two different methods, either by biolistic methods, which is performed using particle gun, or by agrobacterium tumefactions mediated r transformation method. Recombinant DNA methods have facilitated the construction of specific enzymes that align with certain food processing conditions (Ghoshal, 2018). For example, the enzyme Alpha amylases has been generated with high heat stability to be used for manufacturing high-fructose corn syrups that require this type of enzyme. These enzymes were engineered by modifying DNA sequence in the α-amylase amino acid sequences genes (Katsimpouras et al., 2014; Ranjha

et al., 2022). Many other similar enzymes have been engineered and used in food processing and food preservation manufacturing, and these enzymes historically have been proven to be non-toxic.

The application of biofuel also significantly impacts the agriculture and food system by protecting the environment, providing alternative resources to current fuel, and providing many other advantages (Ranjha et al., 2022). Biofuels are generated from plant and plant-based resources, and they are either bioethanol or biodiesel (Hood, 2016). Both types of biofuels are mainly used for transportation. Bioethanol is primarily made from sugar fermentation of cellulose, usually from maize and sugar cane. Bioethanol is used as an alternative to petrol for transport cars. While biodiesel is made from crops oil of palm, soybean, or rapeseed (Sivakumar et al., 2010). However, providing a substitute for the current fuel is not the only goal for biofuel production; biofuels offer many other advantages, including reducing greenhouse emissions, protecting the environment, improving plant yields, and increasing plant protection against abiotic and biotic stress.

Global warming or climate change is a subject of great worldwide concern, and there is quite evidence that emissions from greenhouse gasses contribute to global warming. The primary sources of greenhouse gasses are fossil fuel and electricity usage. Therefore, providing an alternative plant-based fuel will help to reduce greenhouse emissions. Which ultimately prevents a human from the risk of global warming. Biofuels are also carbo-neural, meaning that carbon dioxide is dragged from the environment during biofuel production. Which results in the consumption of greenhouse gas emissions (zero emissions).

Additionally, biofuels reduce the amount of volatile organic materials because of the oxygenation of ethanol and gasoline, and also no need to add lead. Finally, biofuels are non-toxic and have no risk to human health compared to current fossil fuels. The production of biofuels can also be used to increase plant yields by enhancing the efficiency of light during plant photosynthesis. This can be achieved by incorporating photosynthetic bacteria genes into plants, resulting in more light capture and increased productivity than the light from the sun. To this end, biofuels can be used to improve plant protection against abiotic stress by generating plants that are more resistant to stress and producing plants with genes that make the plant more resistant to pathogens and various types of pests (Sawicka et al., 2020). Thus, the production of biofuels has the potential to contribute to future energy security. However, more research and efforts are needed to be carefully evaluated to ensure the safety and adverse effects of food production or other process activities.

Sustainable Agriculture

Agriculture is the backbone of the food system because it provides a renewable raw material for the food industry and serves as the basis of the integrated economy. Building a strong economy through agriculture contributes to substantial development, which will tend to increase in the future by producing higher quality

renewable raw materials sustainably (Lokko et al., 2018). Then, food security and environmental protection will remain guaranteed (Lokko et al., 2018). Having a strong economy in all sectors will ensure global food security and enhance food nutrition value and public health, which will reduce the impact of climate change. Sustainable agriculture using innovative techniques provides the generation of a concrete economy, a healthy environment, and high-quality foods because sustainable agriculture aims to preserve environmental quality, decrease environmental impact, and maintain economic viability (Anderson et al., 2016). Recently, biotechnology has played a significant role in improving the food industry and agricultural activities regarding the quantity and quality of food and agricultural products (Singh & Mondal, 2017).

The application of biotechnology in agriculture provides a great variety of scientific tools to improve every area related to agriculture, including plants, animals, and microorganisms and offers a promising solution to the development of plant productivity and sustainability (Montagu, 2019; Singh & Mondal, 2017). Examples of some of these approaches include genetic engineering, tissue culture, molecular breeding, and molecular evaluation (Singh & Mondal, 2017). These approaches provide farmers with tools to generate high-quality new varieties of agricultural crops, identify diseases, or help the industry produce high-added value molecules for food and health (Lokko et al., 2018).

To have sustainable agriculture, it is crucial to manage and maintain natural resources wisely; sustainability must be in all areas of the supply chain to guarantee food security (Montagu, 2019). For example, sustainability in raw materials and energy, reducing emissions, eliminating waste, and promoting the economy. Biotechnology offers magnificent opportunities and provides tools for the sustainability of the whole supply chain and agriculture development (Abah et al., 2010), for example, treating wastewater, solid waste, climatic smart food production.,

For this purpose, biotechnology facilitates the enhancement of agricultural practices, specifically selection and breeding, through genetic engineering. Genetic engineering is commonly used to improve crops that are rich in important sources of diet, such as vitamins, proteins, lipids, and carbohydrates (Fiaz et al., 2021; Francis et al., 2017; Ghoshal**,** 2018). Besides, biotechnology applications can be utilized to improve animal productivity, including transgenic dairy cattle to improve the milk quality and muscle growth in cattle, Transgenic swine to reduce fat in swine, and transgenic eggs as bioreactors to enhance poultry productivity, fish bioengineered to increase production and improve antifreeze property of fish (Abah et al., 2010; Fiaz et al., 2021). Regarding microorganisms' transformation of microorganisms, biotechnology using genetic engineering can perform many improvements to microorganisms, including the removal of carcinogenic compounds, the Inhabitation of pathogenic bacteria, and the generation of carotenoids in microorganisms (Abah et al., 2010).

Finally, numerous biotechnology approaches are used in detecting and identifying disease-causing microorganisms, including pathogens, through molecular techniques (Johnson, 2018). Thus, preventing and reducing diseases utilizing the application of modern biotechnology. Genetically engineering (modified) plants

have different applications, including vitamins-rich plants, essential Minerals, essential amino acids, essential phytochemicals, Iso-flavonoids, flavors, amino acids, sweeteners, and DNA vaccines (Johnson, 2018). Rice modification with enhanced vitamin A content is an example of a vitamin-rich plant using genetic engineering. To this end, it is crucial to mention that genetic engineering (GMO) is not the only technique of biotechnology but includes all other engineering techniques that use living organisms or bio-based methods. Those techniques are non-GMO and do not include genetic modification, for example, improving plant yields by increasing their resistance to disease and pests, droughts, and harsh environments or improving the economy (Jauhar & Khush, 2003). Therefore, novel and existing biotechnology must be manipulated to ensure the production of enough food supply, maintain our resources, and meet food security in the future. Biotechnology has great potential to fight the global challenges of food insecurity (Pal et al., 2017).

Food Safety

Food safety is a field in food science that focuses on the safety of food products and ensures that food is safe for human consumption. Food processing involves many steps and unit operations, which convert raw food items or perishable into edible food products with improved quality and shelf life (Johnson, 2018). The production of safe food with high quality requires all processing steps and techniques must be food-grade and free from any risks to human health, including biological, chemical, and physical contaminants (Maryam et al., 2017). Biotechnology provides tools to aid in successful food safety. For example, it is also a diagnostics tool for monitoring food safety, preventing and detecting food-borne illnesses, and verifying the food safety application. Some other approaches are applied for the purpose of pathogen detection and food safety practices (Ghoshal, 2018). For example, developing emerging methods to protect plants against pathogenic bacteria, viruses, and fungi (Anderson et al., 2016; Maryam et al., 2017). In this context, several plant defensins are being used to produce disease-resistant plants; defensins plants are used against fungal infections due to their robust antifungal properties (Anderson et al., 2016).

On the other side, biotechnology is widely used in food industries to produce different products, such as genetically modified food, to enhance taste and yield, increase shelf life, and improve nutritive value. Food Safety is one of the major worldwide concerns. Ensuring the production and provision of safe food. The application of biotechnology in the food industry is used to improve the quality of food products, enhance the nutritional value, and increase the shelf life of food products (Maryam et al., 2017). Genome sequencing of plants is one of the biotechniques that address problems of food safety, human health, and food security (Agrawal et al., 2013). Therefore, biotechnology could help to ensure the production of a sustainable food supply with high-quality and safe products.

Challenges of Using Biotechnology to Enhance Food Security

The worldwide challenges for the coming decades are the growing population, demographic changes, climate changes, lack of resources, and increased gas emissions from the greenhouse (Charles et al., 2014).

Currently, the growing population in the coming decades and how to secure enough food is one of the most global challenges (Charles et al., 2014). The increased population will affect not only the food supply chain but also other resources such as health and education sectors, lands, and jobs. Another consequence of the upcoming growing population is raising the average income and people becoming richer due to the high demand for foods, willingness to pay more to obtain food products, and the tendency of people to change their diets towards healthier foods (Drewnowski & Popkin, 1997). This diet transition will cause a rise in the price of foods with nutritional value. As the average rich people are more, the demand for other resources, such as land, energy, water, goods, and other resources, will cause the scarcity of resources, leading to adverse effects on the environment (waste, water, and soil pollution) (Charles et al., 2014). Another challenge is global climate or environmental changes, which already have negatively affected our food resources by reducing agricultural productivity due to global warming. This global warming will trigger temperature rise and severe weather occurrences (Charles et al., 2014).

Consequently, global warming may cause worse events, such as floods or droughts. In this case, food production, especially plant productivity and livestock, will be severely affected if the problem is left without proper solutions, leading to food insecurity. Since the primary source of global warming is gas emissions from the greenhouse, immediate action is needed to reduce the amount of gas emissions from the greenhouse. This is not an easy task, but immediate action is required to stop the adverse consequences of the gradual temperate rises. This challenge, but the solution is still possible, and agriculture here must play a role in controlling this problem by using biotechnology to overcome greenhouse gas emissions. Finally, the food industry and the amount of waste. The global concern is the ability to increase food production to meet the growing population. However, the challenge here is not about the capacity to increase food production but rather how much we waste, how to reduce the amount of waste, and how much feed goes to livestock. If we reduce or eliminate the waste, then we can solve the risk of future starvation, feed the growing population, decrease the agricultural inputs, and save the environment at the same time. Changing the human daily habit of diet towards more plants instead of meat will also positively affect human health.

Risks of Using Modern Biotechnology Approaches

Meeting the increasing global demands for food, bioenergy, and specialized products while addressing environmental threats presents significant hurdles for agricultural production. Agricultural biotechnology holds promise in facing these

challenges, but it must first handle the ethical and sociocultural concerns to gain widespread public trust and acceptance. Effectiveness necessitates the development of ethically responsible solutions that are socially inclusive, culturally relevant, and communicated to the public transparently (Harfouche et al., 2021).

Health and Ecological Concerns

Genetically improved (GI) foods are neither inherently good nor bad for human health; their impact depends on their specific composition. GI foods enriched with higher iron content can provide health benefits to individuals with iron deficiencies. However, the transfer of genes between species may inadvertently introduce allergenic properties. Therefore, it is crucial to subject GI foods to allergy testing before their commercialization. Additionally, GI foods with potential allergy risks should be clearly labeled. Labelling serves various purposes, including disclosing ingredients for cultural, religious, or consumer information reasons. Ensuring the safety of GI foods and appropriate labeling is a shared responsibility between the public and private sectors. While public authorities must establish and enforce safety standards and mandatory labeling to safeguard public health, the private sector can address other requirements driven by consumer preferences. It is imperative to remove antibiotic-resistant marker genes used in research before commercializing GI foods to mitigate potential health risks, even if they are unproven. The regulation of GI foods, including assessing environmental risks, should be integrated into a country's general food safety regulations. Developing nations may require support from international agencies and donors to establish regulatory frameworks that are tailored to their specific risk factors. These regulatory systems must encompass food safety, environmental impact assessment, compliance monitoring, and enforcement mechanisms, all of which should be adapted to each country's unique circumstances. Given the pace of economic globalization, efforts to achieve a global consensus on biosafety standards are urgently needed. Policymakers and regulators must consider ecological risks associated with GI foods, such as the potential spread of traits like herbicide resistance to non-modified plants, increased resistance in insect populations, and threats to biodiversity resulting from extensive cultivation of genetically improved crops.

Seeds that allow farmers to deactivate specific genetic traits offer a promising solution to prevent unintended cross-pollination (Pinstrup-Andersen & Cohen, 2000). Due to extensive crossbreeding with wild relatives and ancestral species, the unregulated exchange of genetic traits, particularly those responsible for various forms of resistance to pesticides, pests, and plant diseases. Consequently, this process leads to diminished biodiversity among wild ancestral variations of cultivated crops and the emergence of problematic "superweeds" (Nezhmetdinova et al., 2020). Both food safety and biosafety regulations should align with international agreements and reflect society's acceptable risk thresholds, considering the potential benefits of biotechnology. Inclusivity is vital, and it is essential to involve

impoverished communities directly in discussions and decisions concerning technological advancements, associated risks, and the consequences of alternative approaches (Pinstrup-Andersen & Cohen, 2000).

Socioeconomic Risk

Without implementing policies that ensure equitable access to agricultural biotechnology resources, services, and markets for small farmers in developing countries, there is a significant risk of exacerbating income and wealth inequality. In such scenarios, larger farmers are more likely to reap the benefits of early technology adoption, increased production, and reduced production costs (Leisinger, 1999). Consolidating agricultural biotechnology research among a few companies can decrease competition, leading to monopolistic or oligopolistic profit structures, potential exploitation of small-scale farmers and consumers, and the possibility of obtaining preferential treatment from governments. Effective antitrust legislation and enforcement mechanisms are essential to address these challenges, particularly in small developing nations where only a limited number of seed companies operate. International standards regarding industrial concentration must also be developed, as global policies have not kept pace with economic globalization. Additionally, robust legislation is required to enforce Intellectual Property Rights (IPRs), including those related to farmers' rights to germplasm. These rights should align with the agreements established within the World Trade Organization (WTO) and the Convention on Biological Diversity (Pinstrup-Andersen & Cohen, 2000).

Ethical Consideration

A significant ethical issue arises from genetic engineering and the concept of "life patents" as they can be seen as contributing to transforming plants, animals, and microorganisms into mere commercial commodities, stripped of their inherent sacred qualities. This concern carries substantial weight and is far from being an insignificant matter. The utilization of biotechnological seeds also has the potential to reduce the genetic diversity of crop varieties (Nezhmetdinova et al., 2020). However, it is essential to acknowledge that all agricultural practices involve humans intervening in natural systems and processes, and any attempts to enhance crops and livestock inherently entail some level of genetic manipulation. The continued survival of humanity hinges on precisely these interventions (Pinstrup-Andersen & Cohen, 2000).

The responsible utilization of agricultural biotechnology mandates ethical scrutiny involving experts, beneficiaries, stakeholders, and the broader public. The potential benefits of modern biotechnology in food and agriculture and associated

risks and opportunities are primarily explored in initial applications within industrialized country agriculture (Harfouche et al., 2021). This debate is intricately connected with concerns like food safety, animal welfare, industrialized farming practices, and the role of private-sector corporations (Pinstrup-Andersen & Cohen, 2000).

Conclusion

Biotechnology holds great promise for agriculture and the food industry because it can help us to manipulate the production of food for a variety of improvements in the food and agriculture sectors. Biotechnology thus has the potential to increase the availability of food products and provide sustainable agriculture by increasing crop productivity and yields. It, therefore, plays a significant role in food security.

Biotechnology could also help to support the environment by reducing the use of traditional pesticides in agriculture and reducing the continual need for more agricultural land on which to grow crops. In the food industry, the application of biotechnology improves the production and food supply chain by utilizing genetically modified approaches to enzymes, fermentation, and vitamins, increasing crop variety, and increasing plant resistance to diseases (Jauhar & Khush, 2003). Moreover, the application of modern biotechnology in breeding selection ensures increased crop production and variety as well as the production of crops that are resistant to diseases and pests and more tolerant to different environmental conditions. Additionally, biotechnology significantly enhances the nutritional value of various foods. This novel technology can be expanded to even small-scale farmers if provided with biosafety regulations and proper policies to ensure that there are no risks to human health and no adverse environmental effects. Biotechnology has become a leading technology for supporting the future of global food security by providing sustainable agriculture and safe and healthy foods.

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Chapter 2
Microbial Contamination in the Food Processing Environment

Zahra H. Mohammad, Elba Veronica Arias-Rios, Faizan Ahmad, and Vijay Kumar Juneja

Introduction

The presence of unwanted microbes, including bacteria, viruses, and fungi, is referred to as microbial contamination. In other words, it refers to microbial growth and the toxins produced by their metabolisms; an example of microbial toxin production is mycotoxins (Potortì et al., 2020). Microbial contamination is considered the major food industry challenge (Dhewa & Kumar, 2022; Teixeira et al., 2021). Microorganisms present in food processing establishments can contaminate food products, resulting in foodborne illnesses or food spoilage (Teixeira et al., 2021). Furthermore, microbial contamination could lead to significant food spoilage and waste, as well as financial losses for the company (Odeyemi et al., 2020). As the global population grows and the demand for food continues to increase, reducing food waste is of utmost importance (Do Prado-Silva et al., 2022). Based on a study by the United Nations Food and Agriculture Organization (FAO), almost 1.3 billion tons of food are lost annually due to microbial spoilage or waste (FAO, 2019).

Microbial contamination poses a significant risk to food safety, potentially leading to the outbreak of foodborne illnesses. The food processing environment, with

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its complex network of surfaces, equipment, and personnel, presents an ideal breeding ground for microorganisms. Understanding the sources, types, and consequences of microbial contamination is crucial for implementing effective control measures and ensuring safe and wholesome food production.

Sources of microbial contamination in the food processing environment can be attributed to raw materials, water, equipment and surfaces, air, pests, and personnel (Do Prado-Silva et al., 2022). Raw materials, including fruits, vegetables, meats, spices, and dairy products, may harbor pathogens if improperly handled and processed. Inadequate cleaning practices can lead to the formation of biofilms on equipment and surfaces, providing a conducive environment for pathogen and spoilage organisms' growth. Airborne microorganisms can also contaminate the environment, particularly in the absence of proper ventilation systems and control measures (Oliveira et al., 2020). The consequences of microbial contamination in foods can have substantial economic impacts, including food spoilage, product recalls, lawsuits, loss of consumer trust, and increased healthcare costs (Teixeira et al., 2021; Odeyemi et al., 2020).

Adherence to Good Manufacturing Practices (GMPs) is essential for preventing and controlling microbial contamination, ensuring proper employee hygiene, effective cleaning and sanitation protocols, training, and equipment maintenance (King & Bedale, 2017). Implementing Hazard Analysis and Critical Control Points (HACCP) provides a systematic approach to identifying and managing hazards throughout the food production process (Tropea, 2022). Controlling water and air quality through regular testing, treatment, and proper filtration and ventilation systems is also crucial.

This chapter explores the various aspects of microbial contamination in the food processing environment, as well as novel pathogen detection technologies.

Source of Microbial Contamination in Food Processing

There are several ways for microbial contamination to enter the food processing environment (Fig. 2.1). The most frequent ones are contaminated raw materials, contaminated water, food handlers, aerosols, biofilms, pests, inadequate workflow, and cleaning procedures, complex equipment design that leads to improper cleaning and maintenance, and faulty sanitary design of the manufacturing area (Todd, 2020; Gurtler et al., 2014; FDA, 2013; Podolak et al., 2010). According to Reij et al. (2004), a survey conducted by the World Health Organization in Europe highlighted that a considerable portion of foodborne outbreaks in Europe could be attributed to cross-contamination by processing or storage in inadequate locations (4.2%), cross-contamination (3.6%), insufficient hygiene (1.6%), contaminated equipment (5.7%), and contamination by personnel (9.2%).

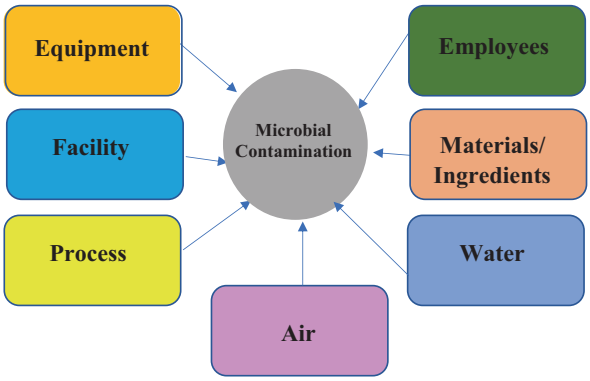

Fig. 2.1 Source of microbial contaminant at food processing

Food Handlers/Operators

Food handlers often play a role in the cross-contamination of food during processing and handling, mainly due to improper hand hygiene practices and lack of training or continued training (Todd et al., 2007; Todd, 2020). Food handlers often transmit infections through contact with the skin, droplets from nasal secretions, stool, or even clothing and shoes (Erdoğan & Pamuk, 2020; Podolak et al., 2010). Training and adhering to GMPs is paramount to preventing contamination of the food processing area and products.

Food handlers can contaminate food by spreading bacteria on surfaces that come into contact with the food, such as worktops and food packaging, before use. They can also contaminate other surfaces in the food processing area, such as door handles, which can facilitate the transmission of bacteria to other food handlers involved in food processing. Several foodborne outbreaks have been associated with poor food-handling practices, including but not limited to, inadequate or failure to wash hands properly and insufficient cleaning and sanitizing of processing tools, equipment, and other food contact surfaces (Todd et al., 2007).

In a large salad dressing recall (Doyle, 2009), consumers reported that the dressing glass jars were exploding. The investigation concluded that the production operator detected a "funny" aroma in one of the drums containing starch paste, however, the operator failed to dispose of the drum and report the finding. Instead, the operator added a small amount of the "funny-smelling starch paste" to product batches for several weeks. Thus, contaminating all the products that had been shipped into the market. A *listeriosis* outbreak linked to soft cheeses was traced back to contamination from farm animals to cheese plant workers. The workers then cross-contaminated the culture solutions used in the cheese production (McIntyre et al., 2015). Fourteen cheese varieties were made with the contaminated starter cultures, causing 48 cases of illness, including three meningitis cases and two cases of bacteremia in pregnancy (McIntyre et al., 2015).

Water

Using contaminated water or ice to wash food, equipment, food contact surfaces, and pack or chill produce is another significant factor contributing to microbial contamination during food processing. (Doyle, 2009). Aside from using water as an ingredient, water is frequently used in food production at different stages, representing 95–99% of the cleaning and sanitizing operation (Bhagwat, 2019). To mitigate the risk of microbial contamination, it is crucial for food processing facilities to ensure the use of clean and safe water sources. Regular monitoring and testing of water quality are also essential to identify any potential sources of contamination and take necessary corrective actions. Furthermore, promoting awareness and providing training to food handlers and processors regarding the importance of using safe water and ice during food processing is vital. By adhering to proper hygiene practices and employing preventive measures, such as using potable water and regularly cleaning and sanitizing equipment, the risk of microbial contamination can be significantly minimized, ensuring the safety and quality of processed food products.

Water plays an essential role in many post-harvest activities, including washing, rinsing, chilling, and general cleaning of fresh produce. Gagliardi et al. (2003) reported that cantaloupe melon rinds often had greater microbial counts than the field-fresh melons harvested in two sites of the Rio Grande River Valley. The aerobic plate count in the former was 2.5–3.5 Log CFU/g, while the washed melons were 4.0–5.0 Log CFU/g.

Fresh-cut produce processing and frozen fruit and vegetable manufacturing are particularly water-intensive practices (Bhagwat, 2019). Reuse of water in post-harvest operations is common to conserve water and energy. However, this practice can lead to cross-contamination between different batches due to the accumulation of dirt, organic matter, pathogens, and chemical residues. It is recommended that post-harvest water in contact with fresh produce, without prior microbial treatment, should meet potable water quality standards. An investigation of a listeriosis outbreak associated with soft-ripened cheese in Canada found that cross-contamination of *Listeria* may have happened with contaminated water used during the curd-washing step. Contamination of the water was traced to the plant's open water reservoir, where wild animals had access, as well as a failure in the facility's water disinfection system (McIntyre et al., 2015).

To ensure the microbiological quality of post-harvest water and minimize contamination and cross-contamination, appropriate disinfection methods should be applied and closely monitored, although this process can be complex and costly (Murray et al., 2017). Numerous instances of foodborne disease outbreaks linked to produce have been suggested to potentially result from cross-contamination occurring during the washing and processing stages, and several studies have been focused on discerning the way contamination can occur (Murray et al., 2017).

Raw Materials

Microorganisms can enter the food processing environment through contaminated raw materials, such as fruits, vegetables, meats, dairy products, spices, etc. Raw materials not further processed through a kill step can contaminate products in later steps in the production line. For instance, in the 2009–2010 multistate outbreak of salmonellosis associated with the consumption of salami, black and red pepper were found to be the source of contamination of salami when applied after the salami's lethality steps (CDC, 2010). In 2007, an investigation of an outbreak of salmonellosis associated with a corn and puffed rice snack concluded that the source of contamination was a seasoning mix added to post-kill heat-treated cereals (Sotir et al., 2009).

Direct contact with contaminated raw materials on equipment surfaces, aerosol production such as powdered spices, or during carcass hide removal, can contaminate the processing environment (Hill, 2015). In the case of fresh produce, contamination with pathogens can enter the food processing plant with dirt on the incoming produce, from extraneous matter, and damaged or decaying units. Later in the processing stream, the quality of the water for washing, disinfecting, and cooling also plays an essential role in the cross-contamination of products and surfaces (FDA, 2008).

In 2020, an outbreak of salmonellosis associated with fruit mix, including honeydew, cut cantaloupe, and cut pineapple, resulted in 165 reported illnesses and 73 hospitalizations. In the investigation, the FDA and the CDC did not identify the source of contamination but found the plant was not maintained clean and in sanitary condition, and there were deficiencies in the company's hazard analysis system (FDA, 2020). Leafy greens were linked to *L. monocytogenes* infections, where 18 people were infected. The FDA/CDC investigation detected *L. monocytogenes* on equipment that harvests iceberg lettuce. Results from the WGS demonstrated that the strains isolated from the facility matched the strain causing illness (FDA, 2021) (Table 2.1).

Biofilms

Microorganisms can persist and contaminate the food processing environment for prolonged periods, contributing to ongoing contamination (Alvarez-Ordonex et al., 2019; Dass & Wang, 2022). Once on a surface, microorganisms can quickly adhere to it, and biofilms can be formed. A bacterial biofilm is a structured community of microorganisms that adheres to inert surfaces and is embedded within self-produced extracellular polymeric substances that eventually create a three-dimensional structure (Alonso et al., 2023). The extracellular matrix, which consists of a combination of polysaccharides, proteins, and nucleic acids produced by the bacteria, acts as a glue-like substance that holds the biofilm together. The biofilm formation starts with

Table 2.1 Recent foodborne outbreaks and implicated food items

Food item	Implicated pathogen	Year	Reported cases	
Raw flour	*Salmonella* Infantis	2022–2023	14	https://www.cdc.gov/salmonella/infantis-03-23/details.html
Ground beef	*Salmonella* Saint Paul	2023	18	https://www.cdc.gov/salmonella/saintpaul-07-23/index.html
Frozen strawberries	Hepatitis A Virus	2023	10	https://www.cdc.gov/hepatitis/outbreaks/2023/hav-contaminated-food/index.htm
Leafy greens	*L. monocytogenes*	2023	19	https://www.cdc.gov/listeria/outbreaks/monocytogenes-02-23/details.html
Eniki mushrooms	*L. monocytogenes*	2023	5	https://www.cdc.gov/listeria/outbreaks/enoki-11-22/index.html
Peanut butter	*Salmonella* Senftenberg	2022	21	https://www.cdc.gov/salmonella/senftenberg-05-22/details.html
Fully cooked chicken	*L. monocytogenes*	2021	3 (1 death)	https://www.cdc.gov/listeria/outbreaks/precooked-chicken-07-21/index.html
Cut fruit	*Salmonella* Javiana	2020	165	https://www.cdc.gov/salmonella/javiana-12-19/index.html
Flour	*E. coli* O26	2019	21	https://www.cdc.gov/ecoli/2019/flour-05-19/index.html
Ice cream	*L. monocytogenes*	2010–2015	10 (3 deaths)	https://www.cdc.gov/listeria/outbreaks/ice-cream-03-15/index.html

a reversible attachment of planktonic cells on a surface. Then, the organisms become irreversibly attached within seconds or minutes. Once connected, they multiply in a matter of hours to produce the extracellular matrix. Finally, secondary colonizers attach and form part of the biofilm. Bacteria within the biofilm may communicate by quorum sensing to interact with each other and even exchange genetic material (Alonso et al., 2023). This matrix provides structural support and protection to the bacteria within the biofilm against cleaning and disinfection processes, making the removal challenging (Shi & Zhu, 2009; Carrascosa et al., 2021). To overcome the effect of the toxic substances, the bacterial strains activate membrane permeability mechanisms and up-regulation of efflux pump activity to eliminate these harmful compounds (Karatzas et al., 2007; Soumet et al., 2012). Additionally, strains isolated from biofilms have shown not only strong tolerance to disinfectants but also cross-tolerance to other sanitizers that may be increased by activation of stress genes, sublethal exposure to treatments, senescence, and production of stress-related proteins (Beuchat et al., 2011; Condell et al., 2012; Soumet et al., 2012).

Biofilm formation in food processing facilities is more frequently associated with humid environments, and a common misconception is that biofilms are less likely to form in low-moisture environments. The truth is that dry environments are also susceptible to biofilm contamination, and even if no water is observed, small amounts of water can be present at the microscopic level in the form of microdroplets and thin liquid films (Gurtler et al., 2014). This can allow the formation of dry surface biofilms (DSB). Similar to wet surface biofilms, eradicating DSB represents a challenge for the low-moisture food industry. However, an additional problem for biofilm management and control in low-moisture food environments is that sanitation should be done without the presence of water (Alonso et al., 2023). Some studies have reported that DSB is less susceptible to stressors such as heat, high pressure, and disinfectants than hydrated films (Beuchat et al., 2011; Almatroudi et al., 2017; Rahman et al., 2022). Microorganisms such as *Micrococcus* spp., *Staphylococcus* spp., *Bacillus cereus*, *E. coli*, *Shigella*, *S. aureus*, *Listeria monocytogenes*, *Pseudomonas*, *Vibrio*, *Cronobacter sakazakii*, *Geobacillus stearothermophilus*, and *Anoxybacillus flavithermus* are common biofilm-forming microorganisms that can easily adhere to equipment and processing surfaces in the food processing environment or even food (Beuchat et al., 2011; Carrascosa et al., 2021).

Preventing the formation of biofilms on surfaces is crucial to controlling microbial contamination. Good production hygiene, proper maintenance and cleaning practices, and well-designed cleaning and decontamination processes effectively limit microbial growth. The hygienic design of equipment and process lines plays a vital role in preventing biofilm formation on contact and non-contact surfaces. Attention should be given to dead spaces, corners, crevices, cracks, gaskets, seals, valves, fasteners, and joints, as these areas can harbor microorganisms and affect process conditions.

Aerosols

Airborne contaminants have the potential to infiltrate processing areas through various entry points, such as doorways, hatches, drains, and other routes when contaminants are carried from raw materials, personnel, rotating equipment, and animal handling (e.g., dehiding carcasses) (Burfoot et al., 2003). Contamination of other areas occurs, especially if the facility does not count with prevention measures such as positive pressure in the processing areas, restricting the airflow from dirty to clean areas, and air filtration. In 2008–2009, the investigation of the salmonellosis outbreak associated with white pepper found widespread *Salmonella* contamination in the facility where the spice was processed and packed. The incoming whole white pepper was found to be contaminated with *S.* Rissen, and during grinding, spice dust contaminated other areas, including the packing area (FDA, 2013). Inadequate cleaning operations have also been related to sources of airborne contamination in food processing plants (Burfoot et al., 2003).

Pests

Microbial contamination in the food processing environment can occur through various sources. Understanding these sources and implementing preventive measures, such as maintaining good hygiene practices, improving equipment design, and ensuring proper cleaning and decontamination processes, are essential for controlling microbial contamination and ensuring food safety. Some products, such as spices and grains, are expected to contain a certain degree of foreign material due to the environment in which they are produced and harvested (Paxson, 2019). Filth adulteration is an example of pest contamination. The contamination includes mice, rats, or hairs and excreta from other animals or insects, insect parts, and any other materials. The presence of such filth contamination causes food to be adulterated (Paxson, 2019).

Sanitary Design of Equipment, Food Processing Flow, and Food Processing Facilities and Their Location

Food manufacturers commonly fail to understand that microorganisms can be migrated from crevices or cracks to the food processing environment. Ideally, food processing plants and equipment are built according to sanitary design standards (Lelieveld et al., 2014). A sanitary design of a processing facility consists of a set of standards that must be followed, including equipment designs, structural repairs, changes, purchases to mitigate the potential cross-contamination, pest infestation,

and facility cleaning practices. However, it is not uncommon for food processing facilities that were adapted for processing products and that lack a proper sanitary structural design or a sanitary production flow.

In 2012, the CDC investigation of a salmonellosis multistate outbreak associated with pet food found that the facility did not have hygienic equipment, insufficient hand washing and sanitizing stations, and the equipment had been repaired with duct tape and cardboard (CDC, 2012). Complex processing machines and surfaces can harbor microbes due to the accumulation of residues and the difficulty of cleaning thoroughly (Hua et al., 2019). Moreover, studies have demonstrated that microbial biofilms of organisms, including pathogens, can survive in a challenging environment with little to no nutrition when exposed to chemicals and other stressors on processing equipment, crevices, belts, and floor drains (Zhao et al., 2016). Then, during the production process, the movement and vibrations of the machine components could provide a pathway for organisms to reintroduce themselves into the product. Biofilms can form on contact and non-contact surfaces, providing a persistent source of contamination. In 2008, an investigation of a *L. monocytogenes* outbreak linked to deli meat from a Canadian company found the meat slicers were uncleanable surfaces and were most likely the cause of contamination of the RTE product (Weatherill, 2009). Poor-quality welded equipment, porous materials, corrosive materials, and smooth surfaces can harbor food particles and moisture that can promote biofilm formation.

Poor equipment and process design, traffic flow, ingredient handling, and storage were associated with the cause of roasted oat cereal contamination in an outbreak of salmonellosis in 1998 (Breuer, 1999). Equipment in food production should be designed, fabricated, and installed following hygienic standards that permit easy and adequate sanitation. The design includes a selection of non-porous, smooth, nonabsorbent, nontoxic surfaces free of crevices and cracks (Faille et al., 2018). Food contamination due to poor sanitary design of equipment or infrastructure is not limited to pathogens. Numerous incidents on a large scale have been related to spoilage microorganisms contaminating products because of poor hygienic design of equipment or inadequate sanitation frequency. The production flow from raw ingredients to the final product should be unidirectional whenever possible. Positive airflow should be established from the clean side of the processing area to the "dirty" side (Beuchat et al., 2011).

Microbial Detection Methods in the Food Processing Environment

Traditionally, regulatory approaches to food safety primarily focused on end-product testing and inspections. However, this reactive approach had limitations in detecting and preventing contamination at earlier stages of the food production

process. Recognizing the need for a more proactive and preventive approach, regulatory agencies and food safety organizations have shifted their focus toward environmental monitoring. This involves systematically monitoring and analyzing the food processing environment, including surfaces, air, water, and equipment. By implementing robust environmental monitoring systems, food processors can identify and address potential sources of contamination, improve their overall food safety practices, and meet the evolving regulatory expectations to ensure the production of safe and high-quality food products.

Several techniques are employed to detect pathogens in food processing. These techniques aim to identify the presence of pathogenic microorganisms and assess the level of contamination and the presence of microbial indicators and spoilage organisms. The time required to obtain results, sensitivity, and specificity from the different techniques is often tried to be improved as new technologies arrive. In traditional methods, samples collected from various surfaces, equipment, or food contact areas are cultured on specific nutrient media, which often requires a lengthy process until results are available (Law et al., 2015). The presence and growth of specific pathogens can be identified based on their characteristic colony morphology, biochemical, and serological reactions. Due to the time-consuming nature of these steps, timely corrective actions or product releases may be hindered, rendering the traditional approach insufficient to meet the escalating demands for rapid food testing.

A wide array of new technologies has been developed to rapidly detect and identify pathogens, spoilage organisms, and microbial indicators. These innovative approaches include nucleic acid-based, immunological, biosensor-based, and colorimetric methods in conjunction with conventional methods (Law et al., 2015; FSIS, 2021). All new assays used for the detection or enumeration of organisms should be evaluated for the performance in validation studies following the guidelines from appropriate validation schemes such as the AOAC International, International Organization for Standardization (ISO), the Association Françoise de Normalisation (AFNOR), and the MicroVal. A vast number of validated pathogen detection methods kits are listed by the FSIS (2021).

Automated systems that employ artificial intelligence to dilute samples, plate them, incubate, and enumerate colonies offer significant advantages to the food industry. In the context of environmental monitoring, where many samples are routinely collected, these automated systems address the bottleneck issue commonly encountered during laboratory processing. Moreover, these systems provide additional benefits, such as the ability to plate multiple serial dilutions on a single plate, resulting in reduced waste of dilution tubes. Furthermore, specific systems can perform real-time enumeration and establish direct connections to reporting devices, further streamlining the analytical workflow. Some of these equipment units can be programmed to perform plating on the agar surface (spiral plating) or even pour plating with or without overlay and using different agars simultaneously.

Nucleic-Acid-Based Methods

Nucleic acid-based methods for detecting microorganisms involve the analysis of specific genetic material, such as DNA or RNA, to identify and characterize the presence of microbial pathogens or indicators (Zhao et al., 2014). Some common nucleic acid-based methods used for microorganism detection include polymerase chain reaction (PCR), multiplex PCR, real-time PCR, reverse transcription PCR, loop-mediated isothermal amplification (LAMP), nucleic acid hybridization, microarrays, next-generation sequencing (NGS), among others. Common DNA targets include virulence factors. For pathogenic *E. coli*, these genes have the Shiga toxins (*stx1*, *stx2*) and its variants, intimin (*eae*), EHEC adherence factor (*efa*), flagellar H7 gene (*fliC*), EHEC hemolysin (*EHEC-hlya*), and IrgA homologue adhesin (*iha*). For *Salmonella, these genes include* the invasion A (*invA*), *Salmonella* pathogenicity island 1 (*hilA*), tetrathionate respiration (ttRSBCA locus), *Salmonella* enterotoxin gene (stn), Type 1 fimbriae (*fimC*), the origin of replication of *Salmonella* chromosome (*oriC*), among others. For *L. monocytogenes, these genes* include hemolysin listeriolysin O (*hly*), invasion-associated surface protein p60 (*iap*), internalin A and B (*inlA* and *inlB*), LmA antigen/delayed-type hypersensitivity protein (*lma/dth18*), among others (Dwivedi et al., 2015).

PCR is the most commonly used molecular-based technique that amplifies specific DNA sequences of microorganisms (Law et al., 2015). This method allows for the detection of microorganisms with high sensitivity and specificity. A specific DNA target is amplified during PCR in a three-step cycling process. First, the double-stranded DNA is denatured into single strands at a high temperature. Then, two primers anneal to specific regions of the DNA strands, and it occurs at 3–5 °C below the melting temperature of the primer. After annealing, the polymerization process is done by a thermostable DNA polymerase at 68–72 °C. Finally, the resulting amplicons are stained with fluorescent dyes and visualized using gel electrophoresis techniques and lateral flow devices (Janagama et al., 2018). PCR has been used for the detection of foodborne pathogens as well as for the detection of food spoilage organisms like *pseudomonas* (Pellissery et al., 2020), thermophilic spore-forming bacteria (Prevost et al., 2010), lactic acid bacteria (Suzuki, 2020, Siegrist et al., 2015), yeasts (Janagama et al., 2018), and other microbial food spoilers. PCR assays are rapid, sensitive, and highly specific; however, they do not distinguish between DNA from dead and alive cells. However, mRNA-based targets have been reported to have a better correlation with the target viability (Keer & Birch, 2003).

Real-time PCR (RT-PCR), also known as quantitative PCR (qPCR), is a powerful molecular biology technique used to amplify and quantify DNA in real-time during the PCR process. Unlike traditional end-point PCR, where the amplified DNA products are visualized after the PCR is complete, real-time PCR allows for continuous monitoring of DNA amplification as it happens. The key feature of real-time PCR is the use of fluorescent dyes or probes that emit signals proportional to the amount of DNA being amplified. The most popular fluorescent dyes are DNA intercalating agents such as SYBR Green and hydrolysis probes. As the PCR

progresses, the fluorescence increases in direct proportion to the amount of PCR product formed. The fluorescence data is collected at each cycle, and the instrument generates real-time graphs (fluorescence vs. cycle number) known as amplification curves. In addition, more than one target organism can be quantified in the same assay. However, the sensitivity may be compromised when compared with singleplex assays. For example, Lopes and Maciel (2019) compared multiplex qPCR with singleplex qPCR to quantify *E. coli*, *S. aureus,* and *Salmonella* spp. in oysters. Their findings showed that the multiplex qPCR had lower sensitivity but higher specificity than the other assays.

This technique does not differentiate between viable and nonviable cells. To overcome this challenge, DNA intercalating dyes (e.g., ethidium monoazide, EMA, or propidium monoazide, PMA) are used to selectively penetrate damaged cell membranes and cross-link the DNA with photoactivation. Then, during the DNA extraction, the dye-linked DNA can be removed (Nocker & Camper, 2006).

Multiplex PCR is based on a simultaneous amplification of more than one locus with a single reaction. In traditional PCR, a single pair of primers is used to amplify a single target DNA region. However, multiple primer pairs are used in multiplex PCR, each targeting different DNA sequences of interest. Multiplex PCR offers several advantages, including the ability to save time and resources by detecting multiple targets in one reaction, minimizing the amount of sample required, and reducing the risk of contamination. However, designing multiplex PCR assays can be challenging due to the need to ensure that each primer pair works efficiently and precisely without interfering with each other; the primer sets should be designed with similar annealing temperatures, and amplicons should be easily distinguished from each other (Dwivedi et al., 2015; Zhao et al., 2014).

It has been reported that multiplex PCR can detect two to five or more organisms simultaneously (Chen et al., 2012). This technique has been successfully used for the detection of *L. monocytogenes* and *Listeria* spp., *Salmonella* and *E. coli* O157:H7, *Salmonella* and *C. sakazakii* from powdered infant formula and environmental samples, wine and beer spoilage organisms, dairy spoilage organisms, and many more. These tests can handle up to six to nine species in one reaction tube (Suzuki, 2020; Janagama et al., 2018). Hodzic et al. (2023) detected *B. cereus, C. jejuni, E. coli* O157:H7, *L. monocytogenes, Salmonella* spp., *Shigella* spp., *S. aureus,* and *Y. enterocolitica* in artificially inoculated meat products, eggs, cheese, fish, fruits, and vegetables using qPCR with an efficiency of at least 91.2%.

Reverse Transcription PCR (RT-PCR) is a molecular biology technique that combines reverse transcription (RT) and polymerase chain reaction (PCR) to amplify and detect RNA molecules. This technique allows researchers to convert RNA into complementary DNA (cDNA) and then amplify specific regions of the cDNA using PCR. RT-PCR is widely used to study gene expression, detect RNA viruses, and analyze RNA samples.

Loop-Mediated Isothermal Amplification (LAMP) is a molecular biology technique used to amplify specific DNA sequences rapidly and efficiently. Unlike conventional PCR methods, LAMP does not require thermal cycling with multiple temperature steps. Instead, it operates at a constant temperature, typically between

60 and 65 °C. The LAMP technique utilizes a unique set of primers that recognize multiple target regions on the DNA. These primers comprise four to six oligonucleotides, including two outer and inner primers. The loop structure within the inner primers is a crucial feature of LAMP that enables the amplification process to occur efficiently and rapidly. The loop allows the primers to initiate strand displacement, creating a stem-loop structure that promotes repeated amplification cycles. The LAMP reaction involves denaturation, annealing, extension and DNA synthesis, amplification, and detection. The amplicon can be detected visually through turbidity, fluorescence, or color change (e.g., paper-based analytical devices). Due to its simplicity, rapidity, and cost-effectiveness, LAMP has gained popularity for various applications, particularly in resource-limited settings (Nnachi et al., 2022).

Pulsed-field gel electrophoresis (PFGE). PFGE is a robust genotyping method that is utilized for the isolation of large DAN, including the whole genomic DNA, after treatment with a distinctive restriction enzyme. PFGE technique consists of separating the intact chromosomal DNA after lysing bacterial cells implanted in an agarose plug to avoid the mechanical shearing of DNA during the extraction (Matushek et al., 1996). Then, the extracted DNA chromosome within the agarose plug is treated by a unique restricting enzyme to generate more than 12 high molecular weight DNA pieces (fragments). Finally, the DNA fragments are subjected to additional separation through a gel matrix under an electric field between pairs of electrodes. This will aid in separating DNA pieces based on the size since different sizes migrate at different speeds and different times through gel pores of the electric field toward the anode. This method allows the analysis of DNA pieces larger than traditional analysis with restriction enzymes because it provides the analysis of the whole bacterial chromosome in a single gel produced by the restriction enzyme. This is important for the analysis of the pathogens and investigation of the genetic profile of microorganisms for epidemiological purposes. (Blanc, 2004).

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) is an analytical technology used to identify and study molecules like proteins and peptides that have been gaining more and more popularity due to its simplicity, high sensitivity, accuracy, and reproducibility (Akimowics & Bucka-Kolendo, 2020). It works by first mixing the sample with a matrix compound. The matrix vaporizes when a laser beam hits the mixture, releasing molecules as ions. These ions are accelerated by an electric field and enter a flight tube. The time it takes for ions to travel through the tube is measured, and this time is proportional to the mass of the ions. The resulting mass spectrum displays ion intensities at different mass-to-charge ratios, providing information about the molecules' masses. MALDI-TOF MS is widely used in proteomics, microbiology, and materials science for its ability to rapidly analyze complex molecules and substances.

In food processing, MALDI-TOF MS plays a significant role in ensuring the safety and quality of food products. MALDI-TOF MS is employed to quickly and reliably identify bacteria, yeasts, and molds in food samples (Aladhadh, 2023). By creating mass spectra from unique protein profiles of microorganisms, technology

allows for accurately identifying different species. This aids in identifying potential pathogens and spoilage organisms that might affect the quality and safety of fermented beverages, honey, dairy products, meat, and seafood. This technology has been successfully used for the identification of *Salmonella*, *Campylobacter*, *Enterobacter*, *Klebsiella*, *Clostridium*, yeasts, and molds in a wide range of foods, including beef, lamb, seafood, dairy products, fresh produce, and baby food (Aladhadh, 2023).

One potential drawback is the initial cost of the equipment. However, the remarkably low consumable costs and the potential to cut labor expenditures may ultimately lead to a decrease in per-sample expenses over the long term, as opposed to alternative methods (Pavlovic et al., 2013; Akimowics & Bucka-Kolendo, 2020).

Next-Generation Sequencing (NGS) is a term that refers to a set of high-throughput sequencing technologies that have revolutionized DNA sequencing. NGS techniques allow for rapid and cost-effective sequencing of DNA. These technologies can generate millions to billions of short DNA sequences (reads) in a single run. NGS has various applications, including whole genome sequencing, targeted sequencing (focusing on specific regions of interest), transcriptome sequencing (RNA sequencing), epigenetic analysis, metagenomics, and more. NGS platforms like Illumina, Ion Torrent, and PacBio have significantly accelerated the pace of genomic research and made large-scale sequencing projects feasible. NGS technologies, such as metagenomic sequencing, enable the comprehensive analysis of microbial communities in environmental samples. These techniques allow for identifying known and unknown pathogens, providing a broader view of microbial diversity and potential risks in the food processing environment (Imanian et al., 2022).

Illumina sequencing is one of the most widely used NGS technologies. It is based on reversible terminator sequencing, also known as sequencing-by-synthesis. In this method, DNA fragments are attached to a solid surface and amplified to create clusters of identical DNA fragments. Fluorescently labeled nucleotides are added one at a time, and the emitted fluorescence is captured to determine the sequence. Illumina instruments can generate a massive number of short reads (typically 100–300 base pairs) in parallel, making them suitable for various applications, including whole genome sequencing, exome sequencing, and RNA sequencing.

Ion Torrent sequencing is based on detecting ions released during DNA synthesis. As DNA polymerase incorporates nucleotides into the growing strand, it releases a hydrogen ion. These ions are detected by sensors, allowing the determination of the DNA sequence. Ion Torrent sequencing platforms are known for their speed and simplicity. They produce shorter reads than Illumina, often ranging from 100 to 400 base pairs. Ion Torrent sequencing is commonly used for targeted sequencing and small-scale genome projects.

PacBio sequencing, developed by Pacific Biosciences, is based on single-molecule real-time (SMRT) sequencing. It involves monitoring the incorporation of fluorescently labeled nucleotides in real time as a DNA polymerase synthesizes a complementary strand. PacBio platforms can generate much longer reads (thousands of base pairs) compared to Illumina and Ion Torrent. This long-read capability

is particularly valuable for assembling complex genomes, characterizing structural variations, and resolving repetitive regions.

Oxford Nanopore Technologies has developed nanopore sequencing, which involves threading a single-stranded DNA molecule through a nanopore and detecting changes in ionic current as individual nucleotides pass through the pore. This technology has the potential to produce highly long reads (tens of thousands of base pairs) and has applications in real-time sequencing of DNA and RNA, as well as in field-based and point-of-care settings.

Each NGS platform has its own strengths and limitations. Researchers select the platform based on the specific goals of their projects, such as genome assembly, variant detection, gene expression analysis, epigenetic studies, and more. The choice of platform also depends on factors like read length, sequencing depth, cost, and computational resources required for data analysis. As technology continues to advance, NGS platforms are becoming even more efficient, accurate, and accessible, driving further progress in genomics and related fields.

Whole Genome Sequencing (WGS) has revolutionized the field of food safety by providing a high-resolution tool for identifying and tracking foodborne pathogens with unprecedented accuracy. WGS involves sequencing the entire DNA content of an organism's genome, allowing for detailed comparisons of genetic information. In food safety, WGS is used primarily for microbial pathogen detection, outbreak investigations, source tracking, and monitoring of foodborne pathogens. In fact, since 2019, WGS has been the standard method for detecting and investigating foodborne bacteria associated with disease outbreaks in the USA (CDC, 2022). The use of WGS has improved not only the faster detection of bacterial pathogens but also has helped identify and connect clusters of disease, especially when paired with pulse field gel electrophoresis (PFGE). In 2015, public health investigators in Washington state connected almost two dozen *Salmonella* clusters that were originally thought to be related to beef products. Initially, a raw beef dish from an Ethiopian graduation party was suspected to be the source of contamination, but soon after receiving samples from the Ethiopian party cluster, pig roasts, and live pig exposure salmonellosis cases started to be reported. PFGE investigations found a particular uncommon pattern between the same contaminated source. After 2 months of intensive investigations, the public health inspectors found that the raw beef was cross-contaminated with contaminated pork at the restaurant where the meat was ground. This outbreak resulted in 192 sick people, more than 523,000 pounds of pork products, and more than 116,000 pounds of whole pigs recalled (CDC, 2021).

Metagenomics is a field of genomics that focuses on studying genetic material collected directly from environmental samples, such as soil, water, food, and more. Unlike traditional genomics, which involves sequencing the DNA of individual organisms, metagenomics consists in sequencing DNA from entire communities of microorganisms (bacteria, archaea, viruses, and other microbes) present in a particular environment. This approach allows researchers to explore the genetic diversity and functional potential of complex microbial ecosystems without the need to culture the organisms in a laboratory. This technology helps improve food safety

because it can measure changes in microbial populations and detect emerging or new risks. For instance, this technology could be used for routine monitoring of raw ingredients. Changes in the microbiome could determine the presence of certain types of microorganisms that could affect food safety or quality (Billington et al., 2022).

Immunological Tests

The immunological techniques for the identification of microorganisms are based on the reaction between antibodies and antigens, such as enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay, enzyme-linked fluorescent assay (ELFA), and immune-magnetic separation (IMS) (Chang et al., 2016; Välimaa et al., 2015). ELISA and lateral flow devices (LFD) are widely used to detect pathogens and their toxins. The limit of detection of these tests is typically between 103 and 107 CFU/mL, which can be reduced when different detection technologies are used (Välimaa et al., 2015). For example, amplicons from nucleic-acid-based methods can be combined with IMS or lateral flow immunoassay to detect organisms from food and environmental samples (Nuchchanart et al., 2023; Li et al., 2015). PCR-ELISA can reduce 100 times the detection limit of *Fusarium verticillioides* in corn when compared with PCR (Aladhadh, 2023). In general, pairing immunological tests with other technologies has been successfully used to reduce the detection limit and the processing time to obtain highly specific and sensitive results (Abirami et al., 2016).

Enzyme-Linked Immunosorbent Assay (ELISA). ELISA is a popular immunological method that utilizes specific antibodies to detect the presence of pathogenic antigens in environmental samples. It provides a quantitative assessment of pathogen contamination and identification, serotype determination, and capability of toxin production. The basic principle of ELISA involves using antibodies and enzymes to create a color change or fluorescence that indicates the presence and often the concentration of the target molecule (Konstantinou, 2017).

The principle of ELISA involves several key steps. The first step consists of immobilizing the antigen (or antibody) of interest onto a solid surface, such as the wells of a microplate. This surface could be made of plastic, glass, or other materials. The antigen is adsorbed or chemically attached to the surface, creating a solid-phase capture system. A blocking step is performed to prevent the non-specific binding of other proteins or molecules to the coated surface. A blocking agent, such as bovine serum albumin (BSA) or milk proteins, is added to the wells. This reduces background noise and ensures that subsequent reactions are specific to the target antigen. Then, the sample containing the analyte of interest is added to the wells. If the analyte is an antigen, a primary antibody specific to the antigen is added. If the analyte is an antibody, a known antigen is added as a capture molecule. After incubation, excess unbound material is washed away. A secondary antibody is used to

amplify the signal. This secondary antibody is linked (conjugated) to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP). This enzyme-conjugated antibody recognizes and binds to the primary antibody if the antigen-antibody complex is present. After washing away unbound secondary antibodies, a substrate specific to the enzyme is added. The enzyme acts on the substrate to produce a detectable signal, usually a color change or fluorescent signal. The amount of signal generated is directly proportional to the amount of analyte present in the sample. The color change or fluorescence is quantified using a spectrophotometer or a specialized ELISA plate reader. The intensity of the signal is then compared to a standard curve generated using known concentrations of the analyte. This allows for the determination of the concentration of the analyte in the sample (Konstantinou, 2017).

Depending on the specific application and the type of analyte being detected, ELISA can be adapted into various formats, including direct, indirect, sandwich, and competitive assays. Each design utilizes the antigen-antibody interaction principle to achieve specific and sensitive detection and quantification of the target molecules (Konstantinou, 2017). The lateral flow immunoassay (LFIA), also known as a lateral flow test or immunochromatographic assay, has a principle similar to ELISA that relies on specific antigen-antibody interactions. LFIA is designed to work as a one-step or two-step process on a paper-based strip, eliminating the need for extensive laboratory equipment and specialized training. The differences between the different ELISA formats are briefly described below.

In direct ELISA, a specific antigen is immobilized onto a solid surface (such as a microplate well). Then, an enzyme-conjugated antibody that binds to the antigen is added. After washing away unbound antibodies, a substrate that the enzyme can act upon is added. The enzyme will convert the substrate into a detectable signal if the antigen-antibody complex is present.

In indirect ELISA, the antigen is immobilized as before, but a non-conjugated primary antibody is used first instead of using an enzyme-conjugated primary antibody. After washing, a secondary enzyme-conjugated antibody that recognizes the primary antibody is added. This amplifies the signal, as multiple secondary antibodies can bind to a single primary antibody.

In sandwich ELISA, the target antigen is captured by an immobilized capture antibody specific to it. Then, a detection antibody, also specific to the antigen, is added. The detection antibody is usually enzyme conjugated. After washing, a substrate is added, and the enzymatic reaction produces a signal (Konstantinou, 2017).

Competitive ELISA is used to detect the presence of an antibody in a sample. A known amount of the antigen is immobilized, and the sample containing the antibody is added. An enzyme-conjugated version of the antigen is also added. The amount of enzyme-conjugated antigen that binds to the immobilized antigen is inversely proportional to the antibody concentration in the sample.

Bioluminescence Methods

ATP/ADP/AMP Bioluminescence. A widely used test in the food industry is the adenosine triphosphate (ATP) bioluminescence assay. ATP is a molecule found in all living cells and is responsible for storing and transferring energy within cells and metabolizes to ADP and AMP (Adenosine diphosphate and adenosine monophosphate, respectively). The ATP test involves collecting the environmental sample with a swab, extracting the ATP, and adding a luciferase enzyme and luciferin. When ATP is present, it reacts with luciferase and luciferin, resulting in the production of light in a bioluminescent reaction. The emitted light from the bioluminescent reaction is measured using a luminometer, which quantifies the intensity of light emitted. The measurement is typically given in Relative Light Units (RLU). The intensity of the light emitted is directly proportional to the amount of ATP present in the sample. Therefore, the higher the signal, the higher the organic matter on the sample (Jay et al., 2005).

Even though this is a non-specific test to determine if microorganisms are present on a surface, it has been used as a quick way to determine the level of cleanliness and sanitation process (Jay et al., 2005). Although the test has been popular in the food industry, misinterpretation of results can create false confidence in the correlation between RLU and microbial concentration after surface sanitation (Hewage et al., 2022; van Arkel et al., 2021; Omidbakhsh et al., 2014). The APT testing provides quick results, allows real-time monitoring, and helps identify areas requiring further attention or improvement. However, it should be used in conjunction with other testing methods to obtain a more complete understanding of the microbial risks in the facility. This method should not be used to indicate the concentration of organisms.

The drawbacks of this test are (1) the inability to identify pathogens; (2) more specificity is needed since the measured ATP can come from microbial cells, organic residues, or non-living sources; and (3) it cannot give accurate concentrations of viable microorganisms.

However, the interpretation and analysis of the obtained ATP measurement are compared to established thresholds or cleanliness standards. If the ATP reading exceeds the set limit, it suggests a higher level of organic matter or microbial contamination on the tested surface. From a different perspective, this is also an excellent tool for detecting organic matter that can promote bacterial growth/biofilm formation (Shama & Malik, 2013). In this regard, testing for ATP, ADP, and AMP increases the sensitivity of detecting organic matter up to 20 times than when testing ATP itself (Bakke et al., 2019).

Easy-to-use self-contained tests, based on bioluminescence technology combined with enrichment periods, have been developed for the in-situ detection of pathogens and microbial indicators. These tests involve an enrichment step that promotes the growth of microorganisms, followed by a specific enzyme detection step (Calderon et al., 2022).

In the case of microbial indicators for quantitative measurements, the testing procedure begins by collecting an environmental sample using a swab. The sample

is then enriched in chromogenic media for a minimum of 6–8 h and incubated using a dry block. Subsequently, an aliquot of the enriched sample is transferred into a detection device containing a lysis reagent with a specific enzyme and ATP (such as β-galactosidase substrate or β-glucuronidase). The specific enzymatic reaction leads to the release of luciferin, which can be quantified using a luminometer. The resulting readings, expressed in relative light units (RLU), are then correlated with the concentration in colony-forming units (CFU) for microbial indicators.

The testing concept for pathogen detection is similar to that of microbial indicators, but the results are qualitative. However, it is essential to note that false positives can occur in pathogen detection if other environmental organisms share similar enzymes with the target pathogens. For example, the hydrolysis of esculin by *Enterococcus* spp. can result in media blackening, which could be misinterpreted as the presence of *Listeria* spp. Further testing using devices that fluoresce in the presence of phospholipase C can be employed to detect presumptive *L. monocytogenes*. Nonetheless, it is still recommended to perform confirmatory testing using reference methods for presumptive positive results (Calderon et al., 2022).

Biofluorescent Technologies for air particle count. This technology is based on laser diffraction to locate particles in the air. When the laser (405 nm wavelength) encounters biological compounds such as nicotinamide adenine dinucleotide and riboflavin, it measures and classifies the scattered light wavelength (Weber et al., 2019; Behrens et al., 2022). When the light is absorbed by fluorescent molecules in the organic particles, fluorescence is emitted at a longer wavelength. In the case of inert particles, the light is scattered with the same wavelength. Similar to bioluminescence technology, biofluorescent technology does not measure colony-forming units; instead, it uses a new unit called autofluorescence (AFU). It also does not differentiate from non-viable organic particles (Weber et al., 2019). The advantages are real-time and continuous testing, instant result reporting, and no need for sample incubation. However, Eaton et al. (2012) reported that false positive results were obtained from certain substances, such as isopropyl alcohol, a typical surface disinfectant. Also, there is a need to correlate the AFU values to colony-forming units (CFU) for the specific working areas and to determine the acceptance of AFU levels that indicate efficient sanitization and disinfection of surfaces. However, these validations are challenging to overcome since the AFU is more sensitive, and traditional methods may not recover viable but not culturable cells, to mention some examples. Another challenge lies in the ability to discern between dead and viable cells.

Biosensors

Biosensors are analytical devices consisting of a biological element and a transducer that transforms and produces a signal when an interaction between the biological element and the target analyte occurs. The biological elements used are nucleic acids, antibodies, cells, and enzymes, and the signals are optical,

thermometric, magnetic, piezoelectrical, or electrochemical (Hameed et al., 2018; Khansili et al., 2018). Immunosensors are the optical biosensors most widely used. The use of biosensor technology has been expanded for quick and on-site detection of spoilage microorganisms and bacterial pathogens in the food industry.

Phage-based Biosensors Technology. Using bacteriophages to target bacteria complemented with conventional detection methods makes this technology a powerful approach for detecting bacterial pathogens in food and the food processing environment (Al-Hindi et al., 2022; Brovko et al., 2012).

Bacteriophages, often simply referred to as phages, are viruses that infect and replicate within bacteria. Most bacteriophages have a complex structure consisting of a protein coat that protects their genetic material. Some phages also have tail-like structures that they use to attach to specific receptors on the surface of the bacterial host (Brovko et al., 2012). Bacteriophages have the advantage of high specificity to live bacterial host infection and can have quick infection cycles that can go as short as 1 h for virulent phages. They are stable under a wide range of environmental conditions, such as variations in pH, temperatures, and exposure to different organic or inorganic solvents, and they even exhibit resistance to proteases (Al-Hindi et al., 2022). These characteristics are advantageous over molecular-based and immune-based assays (Farrokhzad et al., 2015).

In the last decades, several phage-based biosensors have been developed with the incorporation of transducers that can convert the biological interactions between the phages and the pathogens into measurable signals. Some common types of transducers used include optical (e.g., fluorescent and colorimetric signals), electrochemical, Piezoelectric, surface plasmon resonance, microcantilever, and magnetic, among others. The choice of the transducer depends on the specific application, the level of sensitivity, and the type of pathogen being detected (Hinkley et al., 2018; Farrokhzad et al., 2015; Brovko et al., 2012). In recent years, more and more phage-based platforms have been commercialized for the rapid detection of pathogens not only from clinical or environmental samples but also from food samples. In addition, the high specificity and low detection limit allow for shorter incubation times than traditional and even other rapid detection methods. For example, Arias-Rios et al. (2019, 2020) reported the detection of *Listeria* spp. in ice cream and leafy greens with a detection level of 0.2–2 CFU in 25-g samples after a 12-h enrichment. Nguyen et al. (2020) reported the detection of *Salmonella* in raw ground turkey and powdered infant formula in 9.5 and 18.5 h, respectively. In another study, Martins de Aquino et al. (2021) reported a detection time of 9 h for *Salmonella* in chicken meat, sausage, pâté, and chicken nuggets, with a detection limit of 1 CFU per 25 g (Table 2.2).

Paper-based microfluid sensor techniques are highly specific, sensitive, and selective. The technology is based on performing end-point PCR for DNA amplification and a lateral flow device to detect the amplicon. The low amount of volume of 0.001–0.1 mL reduces the risk of contamination. Results from the pathogenic nucleic acid can be detected visually (Reboud et al., 2019).

Table 2.2 Phage-based biosensor assays and techniques and their targeted pathogens

Matrix	Method	Pathogen	LOD	Enrichment time	References
Ice cream	Sample6 BioIllumination™ technology (luciferase)	Listeria spp.	0.2–2 CFU	12 h	Arias-Rios et al. (2019)
Lettuce	Sample6 BioIllumination™ technology (luciferase)	Listeria spp.		12 h	Arias-Rios et al. (2020)
Ground beef	Luciferase, NanoLuc luciferase (Nluc)	*E. coli* O157:H7	5 CFU	9 h	Zhang et al. (2016)
Milk	Phagomagnetic immunoassay	*Salmonella*	1.4 CFU in 25 mL	6 h	Laube et al. (2014)
Poultry products	PhageDx	*Salmonella*	1 CFU in 25 g	9 h	Nguyen et al. (2020) and Martins de Aquino et al. (2021)
Powdered infant formula	PhageDx	*Salmonella*	1 CFU in 100 g	18 h	Nguyen et al. (2020)
Stainless steel	Sample6 BioIllumination™ technology (luciferase)	*Listeria* spp., *L. monocytogenes*	14–16 h	22 h	Banerjee et al. (2018)

Emerging and Novel Technology for Controlling Microbial Contamination in the Food Processing Environment

The growing concern for wholesome food has led to the development of nonthermal technologies in food preservation. Microorganisms contaminate food by following poor hygiene or forming biofilms generally made of single or multiple species, exhibiting higher antimicrobial resistance, and carrying pathogenic bacteria (Galie et al., 2018). It has also been observed that biofilm pathogens exhibit resiliency against antimicrobial agents, which indicates that the food contact surface does not react against the microbes and must change its coating agent (Refaat, 2011; Rossi et al., 2020). To ensure food safety and quality, the quality assurance systems should enable and verify various control measures and their application. Nevertheless, the emerging designs of antibacterial surfaces are opportune to eradicate or reduce microorganisms' adherence. Thermal treatments, viz. sterilization or pasteurization, efficiently reduce the count of microorganisms but negatively affect the organoleptic properties of food and result in the significant loss of thermolabile compounds (Roobab et al., 2018). Hence, various nonthermal technologies such as high-pressure processing (HPP), ultrasound (US), pulsed electric fields (PEF), ultraviolet light

Table 2.3 Nonthermal technologies (NTT) for preventing the microbial contamination of food (Morales-de la Peña et al., 2018)

NTT	Standard conditions	Affected microorganisms	Results
High-pressure processing	500 Mpa, 10 min	Molds and yeasts	Increase total phenols and flavonoids and higher antioxidant capacity
Ultrasound	24 kHz,120 μm, 400 W, 50–58 °C, 0-10 min	*E. coli*	No significant differences in pH, Brix°, titrable acidity, total carotenoids, phenols, ascorbic acid and color between fresh and sonicated juices
Pulsed light	60–240 flashes, 4.8–19.2 J/cm^2	*E. coli*	pH, Brix°, and color exhibited no significant differences with freshwater
Pulsed electric field	35 kV/cm, 1800 ms, 4 μs-pulse width, 200 Hz, bipolar pulses	*L. innocua*	Sensory characteristics and color were similar to fresh juice
Ultraviolet light	18.4 mJ/cm^2	*E. coli*, *L. innocua*, *S. typhimurium*	No significant changes in protein, vitamin C, and antioxidants
Cold plasma	90 kV, air and MA65 gas, 30–120 s	*S. enterica*	Minimal quality deterioration

(UV), pulsed light (PL), and cold plasma (CP) have been in demand during the last decades (Barba et al., 2018). They ensure food safety and better organoleptic attributes (Table 2.3). Due to their efficiency, they are well-known at the industrial level and have emerged as a potential replacement for thermal technologies.

Conclusion

Microbial contamination of foods is one of the food industry's significant challenges and great concerns because it can happen at any point of the food chain, including pre- and post-harvesting, processing, packing, transportation, and distribution. Microbial contamination mainly occurs due to the unintentional introduction of microorganisms to foods directly or indirectly through food contact surfaces or non-contact surfaces. Therefore, contamination can occur through many routes or sources. Understanding the sources of contamination is a critical step in mitigating and preventing microbial contamination. Traditional approaches have been shown to be limited in detecting, controlling, and preventing microbial contamination. The food industry needs innovative and emerging technologies to mitigate microbial contamination. This chapter covered the most recent and novel methods for identifying the source of microbial contamination in food processing, detection methods, and emerging and innovative technologies for controlling microbial contamination in food processes.

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Chapter 3
Use of Microbe Free Contact Surfaces to Control Food Spoilage: A Step Towards New Food Technologies

Shaibi Saleem, Faizan Ahmad, and Shams Tabrez Khan

Introduction

Food safety is essential not only to meet the nutritional requirement but also to ensure its safe supply to needy people (Ahmed et al., 2022). Consumers are becoming increasingly aware and health conscious therefore the demand for healthy, safe, and good-quality food is increasing (Rawat, 2015; Odeyemi et al., 2020). In recent years due to the busy lifestyle, the demand and consumption of readymade foods have increased tremendously. To satisfy the expectations of the market the food sector is striving to develop methods for delivering safe, fresh, and healthy food to consumers (Albrecht & Smithers, 2018). Food processing is a group of techniques used to turn raw food into value-added food (Ravindran & Jaiswal, 2016). To produce branded, marketable food with consistency of quality a continuous supply of crops and animals is required (Drouillard, 2018). The concept of processing food started during prehistoric times, which includes roasting, smoking, steaming, and baking. Before industrialization, methods like canning and salting were also developed (Ghoshal, 2018; Joardder & Masud, 2019). Food processing techniques are shaping the modern world by ensuring food supply not only to general consumers but especially to space scientists, the army, and other explorers. (Hammond et al., 2015). These techniques help to increase the food shelf life and decrease food waste (Aday & Aday, 2020; Han et al., 2018). After the production of good quality food, its preservation is a major concern for the producers as from production to preservation the food is exposed to various

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factors and its quality may quickly deteriorate due to chemical, physical, or microbial changes (Mastromatteo et al., 2010; De Corato, 2020; Zhao et al., 2022). Especially when the food comes in contact with various surfaces it may be exposed to such threats. Food may get contaminated when it comes in contact with surfaces carrying microbes bringing changes in food quality like off-flavor, loss of nutrients, discoloration, and deterioration of texture (Francis et al., 2012; Giannakourou & Tsironi, 2021; Uebersax et al., 2022). This may result in food spoilage which poses a widespread and significant threat to food security (Pitt & Hocking, 2022; Mc Carthy et al., 2018; Saeed et al., 2019; Singh et al., 2022; Wang et al., 2017; Yang et al., 2017; Zhao et al., 2022). At any given period during manufacturing and storage, food products may either carry a unique microbiota or is susceptible to growth by a certain group of microbes, which depends on their physical and chemical properties. Hence one can predict which bacteria may grow or predominate in a particular food (Anagnostopoulos et al., 2022; Ferrocino et al., 2022; Manthou et al., 2022).

The spoilage may be caused by the growth of an organism in food or through the formation of unfavorable metabolites and toxins (Boziaris & Parlapani, 2017). Microbial spoilage is the primary cause of food degradation, which needs to be controlled if the goal of food security is to be achieved (Gil et al., 2015). Microbes of all kinds including bacteria, protozoans, fungi, and viruses are responsible for food spoilage (Trevanich, 2022). Various factors include pH, temperature, moisture, and oxygen levels. Influence the growth of bacteria in food (Cheng et al., 2022; Perumal et al., 2022). The presence of pathogenic microbes causes what is known as foodborne illness and the toxins produced from microbes cause food intoxication along with financial losses (Abebe et al., 2020; Fung et al., 2018). Food-borne illnesses are a significant burden and challenge for public health around the globe (Cissé, 2019; Todd, 2020). Mycotoxins and parasites that spread through food are typically a problem in developing nations (Grace, 2017). Pathogens like *Salmonella, Listeria monocytogenes, E. coli,* and other pathogenic bacteria pose a health risk associated with food (Balali et al., 2020). Food industries need to minimize the chances of food contamination to ensure the delivery of safe food to consumers (Singh et al., 2022). In South-East Asia, 150 million cases of food-borne disease and 175,000 mortalities due to food-borne illnesses were reported in 2010. And 40% of this burden was carried by children under the age of 5 (Vidhubala Priskillal, 2019). An estimated 50% of the malnutrition is not caused by a lack of food or a poor diet but is rather due to insufficient availability of water and lack of proper sanitation facilities, lack of hygiene, inappropriate handling techniques, cross-contamination of cooked food with raw food and inadequately washed food, which can result in diseases and infections like diarrhea (Ekici & Dümen, 2019).

Since contamination through food contact surfaces (FCS) is a significant threat the decontamination of these surfaces is necessary to improve food health. Decontamination is the process of minimizing or eliminating microbes from objects, surfaces, devices, and environments so that they cannot contaminate food (Mota et al., 2021). Heat, steam, chemical solutions, gases, radiations, and several other techniques including high hydrostatic pressure, ultrasound, pulsed electric field, and pulsed light are used to prevent surface contamination (Sipos et al., 2021). These

techniques help to reduce the chances of contamination but have some unavoidable disadvantages also. The approaches are limited due to safety issues, the sensitivity of the materials that need to be sterilized, a lack of effectiveness, and financial considerations.

Cold atmospheric pressure plasma technology enables multi-target microbial inactivation on surfaces, offering a promising non-thermal alternative to conventional techniques (Dasan et al., 2017; Rifna et al., 2019). These plasma processes provide a special combination of strong reactivity at moderate temperatures because of the non-equilibrium plasma discharges' non-thermal features, which is advantageous for treating temperature-sensitive substrates (Dasan et al., 2017). Non-thermal atmospheric pressure plasmas are used in a variety of applications like electrochemical sensors, preparation of functional surfaces, inactivation of micro-organisms in food and food contact surfaces, preparation of ready-to-eat food, biofilm degradation, and healthcare, etc. (Alonso et al., 2022). This chapter discusses the food contact surfaces and spoilage of food. Different procedures adapted for the decontamination of the food and the emerging technology.

Food Contact Surfaces (FCS) and Their Contamination

Food production involves various processes from production to the consumer level (Saini et al., 2021). During its production, the food comes in contact with various surfaces generally referred to as food contact surfaces (FCS; Addo Ntim et al., 2015). Examples of these surfaces include utensils, cutting boards, flatware, tables, highchairs, microwave oven, and refrigerators (Menini et al., 2022). The FCS is often contaminated by dirt, allergen, and pathogenic micro-organisms, and therefore to avoid contamination of food these surfaces must be cleaned and so it's better to sanitize (Rutala & Weber, 2018). More than 250 sources of foodborne illnesses have been found (Hassan, 2022). Several food quality standards have been implemented globally due to the rise in foodborne infections and illnesses (Maragoni-Santos et al., 2022). The microorganisms that attach to plant and animal tissue can affect food safety and spoilage (Chitlapilly Dass & Wang, 2022). Biofilms are developed on the surfaces, resulting in contamination (Sharma et al., 2022). The quality of food is at risk at each step of its production from harvesting or slaughtering to packaging till it reaches consumers (Kumar et al., 2022). Food spoilage is caused by a variety of microorganisms and is characterized by the emergence of off flavors, deterioration of texture, loss of nutritional components, discoloration, etc. (Giannakourou & Tsironi, 2021). The concern about the bio-deterioration of food is increasing in the food industry (Pandey et al., 2022). It is necessary to find effective and economic techniques, that are easy to use and do not affect the taste and nutritional value of food (Liu et al., 2022). Various raw foods like fish, meats, and poultry are prone to the growth of pathogenic bacteria which get transmitted to cooked, ready-to-eat and fresh food (Gálvez et al., 2010). Additionally, the food may contain certain toxins, spores, and allergens. The transfer of contamination can also occur through the

dispersal of biofilm especially form contact surfaces (Sharma et al., 2022). Foodborne illness is the result of low maintenance, and poor hygiene during food preparation (Rifat et al., 2022). Sometimes using the same cooking utensil for both raw and ready-to-eat foods may lead to contamination (Sharma et al., 2022). The contaminated surface is responsible for the pathogen transfer commonly in domestic settings for example, the transfer of pathogens from raw meat to fresh fruits and vegetables after using the same contaminated equipment (Chea et al., 2022). Low maintenance during the storage of food (refrigerators, Ovens), and poor hygiene during food preparation (unwashed utensils, reuse of same surfaces for different foods) are some of the reasons behind foodborne illnesses (Schirone et al., 2019).

Microbial Risk Associated with Food at Different Levels of the Food Processing

The spoilage or contamination of food by undesirable microorganisms is one of the main threats to food (Misiou & Koutsoumanis, 2021). Food spoilage is a process that results in unfavorable or unsuitable changes to food, such as changes in flavor, smell, appearance, or texture, rendering it unfit for human consumption. And very often this occurs as a result of the biochemical activity of microorganisms (Sikorski et al., 2020). Microbes are the main reason behind food spoilage making it necessary to take preventive measures to control microbial growth in food (Gil et al., 2015). Bacteria, mold, and yeasts may cause various types of food spoilage depending upon the type of food, types of nutrients in food, its moisture content, pH (acidic, neutral, or alkaline), and oxygen levels, etc. (Azad et al., 2019). Food spoilage can occur due to microbial contamination at any stage of food processing or supply chain, like during food packaging, food storage, or transportation. During these processes, food may come in contact with microbes or pathogens if the contact surfaces are not sterilized. Contamination or spoilage of food results in various health hazards including food poisoning, nausea, and diarrhea. (Mohammad et al., 2018).

Contamination of Food by Microbial Biofilm

Poor hygiene during food preparation is a major contributing factor to foodborne illnesses (Kamboj et al., 2020). The ability of microorganisms to the attachment to plant and animal tissue found in food is the first step toward their growth and multiplication in food (Rawat, 2015). Several pathogenic microorganisms, including *E. coli, Campylobacter, Listeria, Salmonella, Klebsiella*, and *Pseudomonas* species pose serious hazards to food especially due to their ability to form biofilms on ready to eat, and on minimally processed food (Ugwu et al., 2022; Giaouris & Simões, 2018). The formation of biofilm on the solid material by micro-organisms is a four-step process as shown in Fig. 3.1 and described below (Myszka & Czaczyk, 2011).

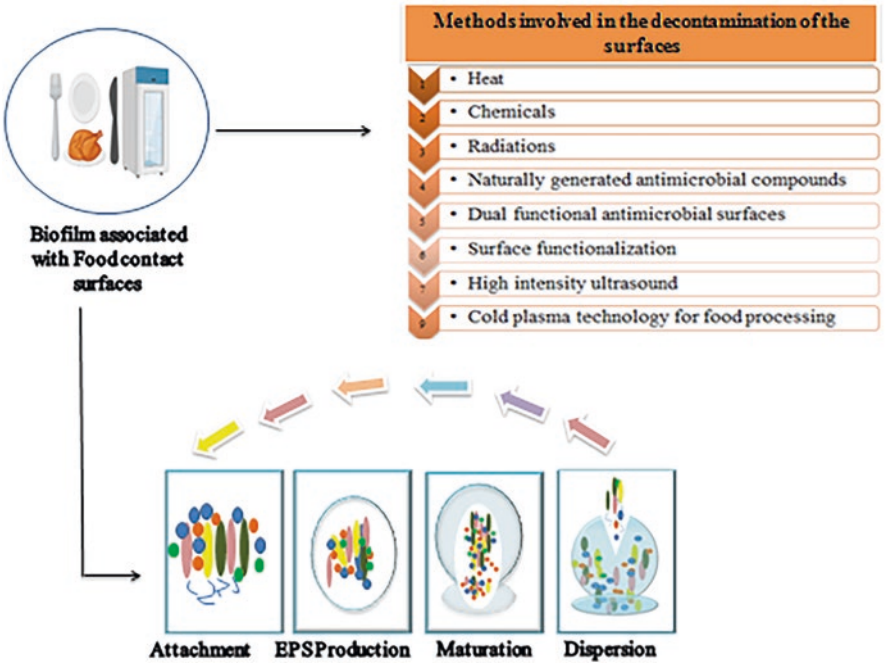

Fig. 3.1 Process of biofilm formation on the food contact surfaces and possible methods for its eradication

(i) Planktonic microorganisms can reversibly attach to solid surfaces.
(ii) The development of biofilm's architecture and the transition from reversible to irreversible adhesion induced by bacteria producing extracellular polymers (EPS).
(iii) Maturation of micro-colonies in a mature biofilm.
(iv) Dispersion of cells from biofilm into the surrounding environment.

Single cells connect to abiotic surfaces to start biofilm formation (Okshevsky & Meyer, 2015). It is possible to generally divide this time-dependent process into two phases: the reversible phase and the irreversible phase. Van der Waals and electrostatic forces cause bacteria to adhere to surfaces within a range of 2–50 nm, at the beginning of the reversible adhesion. Because the majority of bacteria are negatively charged, the negative charge on the surface will lead to electrostatic repulsion. Whereas in the irreversible phase, forces like hydrophobic, dipole-dipole, ion-ion, ion-dipole, covalent bonds, and hydrogen interactions are also involved in interaction with surfaces (Myszka & Czaczyk, 2011). The bacterial attachment to the surfaces and detachment from such surfaces is a survival strategy by the bacteria's host environment because the attachment to host surfaces is advantageous for microbes as it provides nutrients to the microbes minimizing competition for nutrients

(Abebe, 2020). But on the contrary, the microbes may also have the mechanism to evade protective measures taken by the host against such microbial attachment. In the same way, detachment may promote the movement of the bacteria to another potential host surface especially when the environmental condition becomes unfavorable. The maturation of the biofilm starts once bacteria have irreversibly bound to a surface (Berne et al., 2018). The communication between a right quorum of the bacterial population favoring a strong biofilm is facilitated by AHLs (Acylated homoserine lactones) in Gram-negative bacteria and by peptides among Gram-positive bacteria. These act as signaling molecules for cell-to-cell communication regulating population density and gene expression to control the development of biofilms and the release of cells (de Dieu Habimana et al., 2018). The nutrients used by the cells to grow and divide are obtained from the fluid environment around them. As a result, micro-colonies begin to grow and merge into layers of cells that blanket the surface. According to the cultural conditions, the biofilm takes several days to grow in terms of thickness (Moreno Osorio et al., 2021). As the biofilm ages, the associated bacteria separate and scatter in order to survive and colonize new niches. This is how the bacterial biofilm colonizes the food surfaces and causes foodborne diseases (González-Rivas et al., 2018). Various disinfectants for surfaces and modified antimicrobial surfaces are being used nowadays to protect food contact surfaces from potential pathogens. Some microorganisms like, *L. monocytogenes* form biofilms that are so strong that they cannot be removed even after cleaning with disinfectant (Galie et al., 2018).

Food Spoilage by Microbes

Food spoilage is caused by various types of yeasts, mold, and bacteria (Petruzzi et al., 2017). Yeasts are typically known for their ability to raise bread and ferment various alcoholic beverages and can grow in food with or without oxygen (Mani, 2018). They frequently colonize high-sugar or high-salt foods, pickles, sauerkraut, spoiling maple syrup, etc. (Rawat, 2015). Low-pH fruits and liquids are other targets, and some yeast can also grow on the surfaces of cheese and meat. *Candida* spp., *Cryptococcus*, *Debaromyces*, *Hansenula*, *Dekkera/Brettanomyces* spp., *Pichia*, *Phodotorula*, *Torulopsis*, *Trichosporon*, and *Zygosaccharomyces* spp., etc. are the few examples of yeasts responsible for food spoilage (Leyva Salas et al., 2017). Molds often create airborne spores, that may develop effectively on solid substrates in the presence of oxygen. Spoilage molds include species of *Aspergillus*, *Byssochlamys*, *Fusarium*, *Mucor*, *Rhizopus*, *Penicillium*, etc. (Sahu & Bala, 2017). Bacteria can grow at different temperatures and can be grouped as psychrotrophilic, mesophilic, and thermophilic (Le Marc et al., 2021). Generally, thermophilic spore-forming bacteria with the ability to tolerate high temperatures are mainly responsible for the spoilage of canned food (Petruzzi et al., 2017). Bacteria (*Campylobacter jejuni*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*,

Cronobacter sakazakii, *Escherichia coli*, *Listeria monocytogenes*, *Shigella* spp., *Staphylococcus aureus*, *Salmonella spp.*, *Vibrio* spp., and *Yersinia enterolitica*), viruses (*Noroviruses and Hepatitis A*) and parasites (*Toxoplasma gondii, Cyclospora cayetanensis* and *Trichinella spiralis*) are common foodborne pathogens (Bintsis, 2017). Fungi like *Diplodia*, *Monilinia*, *Alternaria*, *Phomopsis*, *Rhizopus*, *Botrytis*, *Pencillium*, *Fusarium*, etc. are the most frequent pathogens that cause rots in fruits and vegetables. *Erwinia, Pseudomonas*, and other bacteria may inflict serious damage to food (Shanmugam et al., 2021).

Foodborne Illnesses

Acute or sub-acute non-infectious diseases caused by the food containing biological agents are referred to as foodborne diseases or food poisoning (Hernández-Cortez et al., 2017). Contaminated food (by pathogens, parasites, viruses, and chemical substances) is responsible for more than 200 diseases from diarrhea to cancers (Bhaskar, 2017). Foodborne illnesses are usually infectious or toxic, many may lead to long-term disability, and death (Sharif et al., 2018). It causes diseases, malnutrition and risks food security, and increases public health concerns. Foodborne illnesses include *Staphylococcal* poisoning, *Vibrio* infection, *Mycotoxin* poisoning, *Enterohemorragic collitis*, Cholera, *Escherichia* gastroenteritis, non-hemorrhagic colitis, and salmonellosis. (Gourama, 2020). Malnutrition affects infants, young children, the elder, and the immunocompromised person (Steiber et al., 2015). The impact of foodborne infections on public health and the economy is reported to make it difficult to establish links between food contamination and consequent illness and death. According to the WHO report (2015) on the disease burden of 31 common foodborne agents at the global and sub-regional levels, it is estimated that 600 million cases people (nearly 1 in 10) worldwide get sick after eating contaminated food and 420,000 die every year. Which corresponds to the loss of 33 million healthy life years). Foodborne diseases have a different impact on different age groups. Children under the age of 5 years constitute 40% of global foodborne illnesses leading to an annual death of 125,000 (Amodio et al., 2022). In addition to burdening health care systems, foodborne infections also harm national economies, international trade, and tourism (Negesso et al., 2016). The WHO observed the prevalence of foodborne diseases in the African region. Over 92 million people get sick each year, and 137,000 die every year (Bisholo et al., 2018). Out of which 70% of foodborne illnesses in Africa result in diarrheal infections. Non-typhoidal Salmonella, which can spread through contaminated eggs and poultry and is responsible for more than half of the global deaths, killing about 32,000 people annually in the Region. *Taenia solium* (the pork tapeworm) alone causes 10% of foodborne illnesses and is a matter of concern (Eng et al., 2015) (Table 3.1).

Table 3.1 Foodborne illnesses, causal agents, symptoms, and epidemiology

Foodborne illness	Microbes involved	Symptoms	Food source	Geographic distribution	References
Bacteria					
Staphylococcal poisoning	*Staphylococcus aureus*	Nausea, vomiting, stomach cramps, and diarrhoea	Eating product contaminated with staph toxin	Most frequent foodborne illnesses worldwide	Zhang et al. (2022), Johler et al. (2015), and Lee et al. (2022)
Vibrio infection/ Vibriosis/Acute gastroenteritis	*Vibrio vulnificus*, *Vibrio cholera*	Watery diarrhoea, vomiting and abdominal pain	Raw or undercooked seafood, rice, vegetables, millet gruel	Worldwide	Dutta et al. (2021), Fung et al. (2018) and Osunla and Okoh (2017)
Enterohemorragic collitis	*E. coli O157:H7, E. coli O26:H11*	Vomiting, fever, headache, nausea, and diarrhoea	Undercooked meat, unpasteurized milk, contaminated fruit and vegetables	Worldwide	Lye et al. (2013), Adley and Ryan (2016) and Heredia and García (2018)
Escherichia gastroenteritis	Enteropathogenic and enterotoxigenic *E. coli*	Diarrhoea, stomach cramps and occasionally fever	Contaminated ground beef or water or unpasteurized milk	High risk areas include Africa, Mexico, and America	CDC (2019), Hazen et al. (2017) and Lääveri et al. (2018)
Bacterial hemorrhagic enterocolitis	*Campylobacter*, *Salmonella*, *Shigella*, enteroinvasive and enterohemorrhagic *Escherichia coli*, *Yersinia*, *Chlamydia*, *Neisseria*, and *tuberculosis*	Bloody, mucopurulent stools that are frequently accompanied by fever, tenesmus, and stomach pain	Ingestion of contaminated food and water	Worldwide	Alharbi et al. (2022), Ganguly et al. (2012) and Dejene et al. (2022)
Salmonellosis	*Salmonella*	Diarrhea, fever, and stomach cramps	Animal origin products	Coastal counties, non-coastal counties	Ame et al. (2022), Chlebicz and Śliżewska (2018) and Godínez-Oviedo et al. (2022)

Campylobacteriosis	*Campylobacter jejuni*	Dysentery or bloody diarrhoea syndrome, which typically includes cramps, fever, and pain	Raw milk, undercooked or uncooked meat, poultry, and shellfish	In Australia, campylobacteriosis is the most commonly reported foodborne infection	Epps et al. (2013), Kaakoush et al. (2015) and Lee and Yoon (2021)
Listeriosis	*Listeria monocytogenes*	The main symptoms include fever, chills, muscle pains, nausea, and diarrhea. Other consequences include miscarriage in pregnant women, infant deaths, it also effect people with compromised immune systems	*Listeria* is present in unpasteurized dairy products, a wide range of ready-to-eat foods, and can survive in refrigerator conditions	Widely distributed in the natural environment	Jackson et al. (2016), Donovan (2015) and Madjunkov et al. (2017)
Viruses					
Food poisoning/ stomach flu/stomach bug/infectious gastroenteritis	*Norovirus*	Abdominal pain, watery diarrhoea, vomiting, and nausea	Consumption of shellfish, such as oysters, direct interaction with an infected individual, drinking or eating tainted food	United States	Gourama (2020), Adley and Ryan (2016) and Ushijima et al. (2014)
Liver disease	*Hepatitis A virus*	Long-lasting liver disease	Raw or undercooked seafood or contaminated raw produce, ingestion of HAV-contaminated food or water	Central and South America, the Middle East, the Indian subcontinent, and Africa	Mohammad et al. (2018), LaRocque and Harris (2021) and Switaj et al. (2015)
Gastroenteritis	*Rotaviruses*	Severe diarrhea, vomiting, fever, and dehydration	Ingestion of contaminated food such as shellfish, salads, or ice	Worldwide	Lai et al. (2020), Stuempfig and Seroy (2021) and Oteiza et al. (2022)

(continued)

Table 3.1 (continued)

Foodborne illness	Microbes involved	Symptoms	Food source	Geographic distribution	References
AstV gastroenteritis	*Astroviruses*	Watery diarrhoea	Contaminated food and water are major sources	Worldwide	Pakbin et al. (2022), Sajewski et al. (2022) and Omosigho et al. (2022)
Gastroenteritis	*Sapovirus*	Diarrheic stools tend to be watery and non-bloody	Eating of raw bivalves such as oysters	Worldwide	Sajewski et al. (2022) and De France et al. (2022)
Parasites					
Cystic echinocccosis	*Echinococcus granulosus*	CE causes harmful, slowly enlarging cysts in the liver, lungs, and other organs	Echinococcus spp., can be transmitted as contaminants of food	Argentina, Peru, East Africa, Central Asia and China	Joanny et al. (2022), Mahmood et al. (2022) and Gabriël et al. (2023)
Taeniasis	*Taenia saginata* (beef tapeworm), *Taenia solium* (pork tapeworm), and *Taenia asiatica* (Asian tapeworm)	Abdominal pain, weight loss, digestive disturbances, and possible intestinal obstruction	Eating contaminated beef or pork	*T. saginata* occur in Eastern Europe, Russia, eastern Africa and Latin America, *Taenia solium* taeniasis is seen in the United States, and *Taenia asiatica* is limited to Asia and is seen mostly in the Republic of Korea, China, Taiwan, Indonesia, and Thailand	Lianou et al. (2023), Owusu-Apenten and Vieira (2023) and Pradhan and Karanth (2023)
Toxoplasmosis	*Toxoplasma gondii*	Symptoms include muscle pain, fever and headache, all of which can last for weeks	Eating undercooked, contaminated meat (especially pork, lamb, and venison) or shellfish (for example, oysters, clams or mussels)	Toxoplasmosis is usually more prevalent, especially in moist, warm and low altitude regions	Kuruca et al. (2023), Montazeri et al. (2020) and Lianou et al. (2023)

Ascariasis	*Ascaris lumbricoides*	Abdominal discomfort or pain	By eating vegetables or fruits that have not been carefully peeled, washed, or cooked	Worldwide	Oniya et al. (2023), Sumner and Peters (2023) and Devleesschauwer et al. (2018)
Gastrointestinal amebiasis	*Entamoeba histolytica*	Diarrhea	Contaminated food	Tropical and subtropical developing countries	Pal (2020), LaRocque and Harris (2019) and Ünüvar (2018)
Prions					
Creutzfeldt-Jakob disease (vCJD)	*Prion* protein	Personality changes, memory loss, impaired thinking, blurred vision or blindness, insomnia, incoordination, difficulty speaking, difficulty swallowing	Consumption of beef products from cattle infected with bovine spongiform encephalopathy (BSE), a condition sometimes referred to as "mad cow disease"	Worldwide	Seed et al. (2018), Forsythe (2020) and Kennedy et al. (2020)
Toxins					
Mycotoxin poisoning/ Mycotoxicosis	*Aspergillus*, *Penicillium* and *Fusarium*, and others include *Alternaria*, *Claviceps* and *Stachybotrys*	Nephropathy, different cancers, alimentary toxic aleukia, hepatic illnesses, hemorrhagic syndromes, immunological and neurological conditions	Mycotoxins can be consumed directly through staple foods or indirectly through animals fed contaminated feed, particularly through milk	Worldwide	Liew and Mohd-Redzwan (2018), Awuchi et al. (2022) and Cinar and Onbaşı (2019)
Ciguatera fish poisoning (CFP)/ Ciguatoxin	*Gambierdiscus toxicus*	Nausea, vomiting, and neurologic symptoms	Consuming certain tainted tropical and subtropical fish	Indian and Pacific Ocean, and the Caribbean Sea	Friedman et al. (2017), Edwards et al. (2019) and Ikehara et al. (2017)

(continued)

Table 3.1 (continued)

Foodborne illness	Microbes involved	Symptoms	Food source	Geographic distribution	References
Paralytic shellfish poisoning/Saxitoxin	*Dinoflagellates*	Vertigo, gastrointestinal problems, and neurological issues paralysis can result in mortality and respiratory failure	Shellfish and other fish	Southern Chile, the North Sea, northwest and northeast United States, and Japan	Hurley et al. (2014), Etheridge (2010) and Ain et al. (2021)
Neurotoxic shellfish poisoning/Brevetoxin	*Dinoflagellates*	Muscle pains, ataxia, with diarrhoea, vomiting, and nausea	Shellfish and other fish	Worldwide	Sinno-Tellier et al. (2023), Abraham et al. (2021) and Estévez et al. (2023)
Puffer fish poisoning/ Tetrodotoxin	The major TTX-producing microbes belong to the genus *Aeromonas*, *Alteromonas*, *Bacillus*, *Pseudomonas*, *Shewanella* and *Vibrio*	Salivation, nausea, and vomiting are common symptoms that might lead to paralysis	Ingestion of contaminated pufferfish	East Asia, specially China and Japan	Al Dhuhaibat and Zarzour (2023), Al Homsi et al. (2022) and Anusha et al. (2021)

Source: WHO report on food safety (2022) https://www.who.int/news-room/fact-sheets/detail/food-safety

Decontamination of Food Contact Surfaces

Decontamination is the process of reducing or eradicating germs from objects, surfaces, and the environment preventing their passage and growth in food (Michels et al., 2015). Surfaces of utensils, cutting boards, flatware, tables, and highchairs which come in contact with food need to be sterilized (Menini et al., 2022). Additionally, it refers to surfaces like the microwave and refrigerator where food may spill, drain, or splash (Owusu-Apenten & Vieira, 2022). Mostly aqueous cleaning solutions are used to remove bacteria on the surface of the equipment. The food industry is using various conventional methods to sterilize equipment surfaces and machines which include heat, chemicals, and radiation (Jildeh et al., 2021). Heat in different forms like hot air, hot water, and steam reduces the number of pathogenic organisms in the food contact surfaces (Sharma et al., 2022). The application of high-velocity steam on the surface can effectively disinfect surfaces (Fukuda et al., 2020). But it only works on surfaces on which the surface is directly exposed and is not effective for concealed spaces. Radiations used for decontamination include ionizing, infrared, and UV radiation. Radiation is used less commonly than heat and chemical treatment due to the high cost (Chauhan et al., 2018). Chemicals like chlorine (reduces the biofilm of *L. monocytogenes*), chlorine dioxide (reduction of *Bacillus cereus* on stainless steel surfaces), iodine, nisin, carvacrol, hydrogen peroxide, quaternary ammonium compounds, and Triclosan (reduce the growth of *Serratia, E.coli*, and *Salmonella*), etc. (Sharma et al., 2022). Iodine and chlorine react with the food and dirt on the surfaces failing to disinfect properly. The chemical agent's temperature, contact duration, and concentration need to be carefully optimized because too high a concentration can be toxic, and too little concentration will only partially decrease harmful pathogens (Sharma et al., 2022). Other emerging technologies for the sterilization of surfaces are discussed below.

Naturally Produced Antimicrobial Compound

Various antimicrobial compounds can be used for the disinfection of surfaces but the overuse of such antimicrobials is already causing the problem of multidrug resistance. Artificial food preservatives used in food are also associated with serious health hazards (Bearth et al., 2014; Bruna et al., 2018). Hence, there is a rising need for natural products that can replace food preservatives (Castro-Rosas et al., 2017). Animals, plants, bacteria, fungi, and algae all can be used as a source of such natural antimicrobial compounds (Gyawali & Ibrahim, 2014). The effectiveness of such natural chemicals from plants has been confirmed in various studies (Sharma et al., 2022). Polyphenols obtained from grape stems inhibit the attachment of *L. monocytogenes* on stainless steel and polypropylene surfaces (Vazquez-Armenta et al., 2018). The casein protein may be effectively cross-linked by tannic acid and the films containing casein demonstrate improved physicochemical properties making

it a potential film for food packaging (Picchio et al., 2018). Gallic acid (3,4,5- trihydroxy benzoic acid) has demonstrated anti-inflammatory, anti-mutagenic, antioxidant, and bacteriostatic activity (against *E. coli*, *Salmonella* spp. *S. aureus* and *C. vinaria)* (Lamarra et al., 2017). Resveratrol shows antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Glaser et al., 2019). Various essential oil has effective antimicrobial properties depending upon the concentration and type of essential oil used for the inhibition or eradication of pathogenic biofilm (Rossi et al., 2022). There are various essential oil studied that can inhibit the pathogens on the FCS including, carvacrol and *Helichrysum italicum* (which can inhibit the biofilm of *S. aureus*) (Bezek et al., 2022). *Thymbra capitata* a natural sanitizing solution can decrease the growth of *S. enterica* and *E. coli* (Falcó et al., 2019). *E. coli* biofilm can be reduced by 93.43% with clove oil and by 82.30% with thyme oil. *Cinnamomum cassia* and *Salvia officinalis* EOs has been shown to remove the biofilm of *S. aureus*. Cinnamon oil, marjoram oil, and thyme oil act as a disinfectant, Eucalyptus oil and cinnamon oil have antimicrobial activity against *S. aureus* and *E. coli*. Lemongrass oil inhibits the biofilm of *E. coli*. Cinnamon oil has antifungal activity against *Aspergillus niger* (Sharma et al., 2020). The chitosan film containing clove oil can be used in food packaging because of its antimicrobial activity (Saadat et al., 2022). EO obtained from *Mentha spicata* L. has anti *Vibrio* spp. activity, and can be used successfully for the preservation of food (Snoussi et al., 2015). EO from *Satureja montana* L. *Thymus vulgaris* L. has antimicrobial activity against *Salmonella typhimurium* and also helps in extending the shelf life of food (Miladi et al., 2016). Ferulic acid (Hydroxycinnamic acid) increases the quality and shelf life of freshly cut apples (Nicolau-Lapena et al., 2021).

Dual Function Antimicrobial Surfaces

The new strategies to eradicate or remove the pathogenic micro-organisms from the surfaces involve the modification of the food contact surfaces to discourage microbial attachment and build-up on food surfaces (Khelissa et al., 2017). Stainless steel and polyethylene are the most studied surfaces because of their widespread use in food processing equipment and packaging (Van Houdt & Michiels, 2010). Ideal antimicrobials should eliminate the microbes, stop them from adhering, or eradicate them if any are already present (Yu et al., 2015). Three types of dual-function antimicrobial surfaces have been developed. These surfaces include those that can kill resist, repel, and release microbes. These surfaces integrating two techniques in one system have been developed because of numerous investigations and research (Chug & Brisbois, 2022; Banerjee et al., 2011; Afewerki et al., 2020). Antimicrobial surfaces based on the combination of bactericidal and microorganism-resistant qualities are known as dual-function coatings (Zou et al., 2021). These surfaces either have a non-biofouling spacer, like a hydrophilic polymer or layer that prevents adhesion, or a significant release of an antimicrobial chemical that is contained in a non-fouling matrix (Yang et al., 2014). Ahmadi and Ahmad (2019) used

the synergistic activity of -interaction and in situ graphene oxide integration to create a durable, active dual-function (antimicrobial/anticorrosive) polyurethane nanocomposite (PUC) coating. In comparison to planar aluminum, the coated surfaces demonstrated sustained anti-corrosive action in 5% NaCl solution and decreased bacterial surface colonization against *S. typhimurium* by 6.5 and *L. innocua* by 4.0 log-cycles (Sharma et al., 2022). Liu et al. (2020) created immobilized lysozyme as a super hydrophobic coating made from sintered silica nanoparticles to provide a dual-functional coating with antibacterial and anticontact capabilities for aluminum surfaces. The most cost-effective method for loading and releasing antimicrobial agents uses multiple layers as a reserve. This method is called layer by layer method (Chouirfa et al., 2019). The innovative FCS demonstrates excellent thermal insulation and ultra-lightweight characteristics (Sharma et al., 2022). Gao et al. (2019) also investigated a composite system made of a multilayer film composed of PVA/PAA and chitosan/heparin. Additionally, the controlled release of anti-microbial substances from the surface limits the colonization of microorganisms and prevents their spread (Kumar et al., 2021).

Surface Functionalization

Various techniques for surface modifications have been developed to improve the inertness and safety of the food contact materials. Different polar groups can be added to the surface using wet solvents, UV light, and adhesion (Fabbri & Messori, 2017; Nady et al., 2011). As a result, these techniques must alter the surface to incorporate a specific functional group. For surface modification. The choice of polymer is based on criteria like elasticity, conductivity, strength, kind of material (synthetic or natural), and degradability (Nemani et al., 2018). Depending on the use of the surface, the immobilization of biomolecules or functionalization of surfaces is taken into consideration (Stewart et al., 2019). To add the necessary amount and variety of the reactive functional group, the surface's functionalization processes must be improved in the second stage.

High-intensity Ultrasound

In recent years, the use of ultrasound in the food business has attracted a lot of attention (Gallo et al., 2018). Ultrasound is regarded as an economical technique in the food industry (Mason et al., 2011). High-intensity, high-frequency sound waves propagating through liquid are used to speed up the cleaning of surfaces submerged in ultrasonically activated liquid (Chemat & Khan, 2011). Ultrasound has is becoming increasingly popular in recent times and has a wide range of uses in chemical reactions and surface conditioning (Azam et al., 2020). Numerous advantages of this non-destructive technique include reliable cleaning, microbiological safety, and

assurance of food quality (Bhargava et al., 2021). But more investigations are needed to make the technology affordable for industrial applications. The technology of ultrasonic cleaning is distinct and incredibly effective as it can penetrate and clean any surface using sound-conducting liquid as the medium (Gallo et al., 2018). It is also capable of cleaning complicated chores like tread roots, tiny surface shapes, blind holes, and others (Mason, 2016). A positive pressure during the compression cycle can force molecules closer together, whereas a significant negative pressure during the expansion cycle can overcome the liquid's tensile strength and cause gaps. The surfaces created via ultrasonography have a porous shape, a large surface area, and good stability. Because of its antibacterial and self-cleaning qualities, the porous matrix can be filled with antimicrobial materials, covered in various types of coatings, and used as a multifunctional surface (Kollath & Andreeva, 2017).

Cold Plasma Technology for Food Processing

Irving Langmuir coined the name "plasma" in 1928 to describe the fourth state of matter, which is an entirely or partially ionized state of the gas. It is widely used in textiles, electronics, life sciences, and food packaging. (Pankaj et al., 2014; Ekezie et al., 2017; Misra et al., 2019). In the past, plasma technology is utilized as a surface-cleaning tool (Thirumdas et al., 2015). It has been used commercially as disinfection agents on medical equipment surfaces made up of heat-sensitive polymers. For its unique benefits, such as zero or minimal influence on substrate materials, plasma technology is employed in the biomedical sector for the cold sterilization of tools and prostheses as well as for various temperature labile materials (Trimukhe et al., 2017; Desmet et al., 2009). Ionization is consistently regarded as the most crucial element in the processing of plasma, followed by other elements such as reaction rate, rate constants, the mean free path, and the electron energy distribution (Thirumdas et al., 2015). Based on reactions, the plasma chemical process can be split into two groups. There are two types of reactions: homogeneous gas-phase reactions (such as the creation of N_3 from N_2) and heterogeneous reactions, in which plasma interacts with a solid or liquid medium (Tiwari et al., 2020). Plasma can be produced by exposing a gas to an electric field, either continuously (direct current field) or at alternating time interval (typically high-frequency field). These energy sources raise the electrons' kinetic energy, which increases the number of collisions in the gas and causes the generation of plasma products including electrons, ions, radicals, and radiation of various wavelengths, including UV radiation (Bogaerts & Neyts, 2018). According to a report, plasma can penetrate 10 μm deep, but UV rays can only reach a depth of 1 μm. This makes plasma useful for killing and inactivating spore forming bacteria (Jaiswal & Sinha, 2015). O_2 plasma demonstrated effective biocidal action on *B. subtilis* and *Clostridium sporogenes*. Plasmas produced at 200 W were sufficient to kill more than 3.5 $\log_{10}$ of *B. subtilis* in 5 min (Moisan et al., 2001). Hence, cold plasma technology can be effectively used for killing microbes on fresh products to increase shelf life (Bagheri & Abbaszadeh, 2020). In

a recent study, it was found that strawberries treated with cold plasma had a 12–85% decrease in the total mesophilic count and a 44–95% decrease in the yeast and mold counts (Misra et al., 2014). Gurol et al., (2012), used low-temperature plasma to treat raw milk to kill *E. coli*. It was observed that applying cold plasma technology against food pathogens (*L. monocytogenes* and *S. typhimurium*) can reduce their growth (Katsigiannis et al., 2021). Various microbes are inactivated by the cold plasma including *E. coli*, *S. typhimurium*, *L. monocytogens*, *A. paraciticus*, *G. liquefaciens*, *A. flavus*, *A. hydrophila*, *C. albicans*, *S. cerevisiae*, *P. agglomerans*, and *S. enteritidis* (Mandal et al., 2018; Birania et al., 2022). Cold plasma technology is very useful in food packaging as it runs all over the surface and successfully helps to sterilize the outer surface during the handling, transportation, and distribution of packaged food (Ekezie et al., 2017; Pankaj et al., 2014; Misra et al., 2019; Roobab et al., 2022). Cold plasma technology modifies the surfaces by adhesive bonding, cleaning, coating, painting, and printing. Cold plasma can be used to sterilize heat-sensitive packing materials like polycarbonate and polythene because of its low temperature.

Public Health Concern

Bio-deterioration is seen as a significant concern for the food business which can be described as any unfavorable alteration in the food by microbes. Which results in a loss in its nutrient content, and change in colour, or texture making food more brittle. Managing microbiological food safety requires a multifaceted approach and addressing the questions of how to establish effective controls without adding to the cost or compromising flavor and nutritional value. The entire food supply chain must be thoroughly studied for effective management of microbial risk. Screening the microbiological load in the finished product typically fails in terms of hazard control because it is impossible to test enough samples to find pollutants at levels that reflect unacceptable health concerns. Pathogenic bacteria (*L. monocytogenes*, *S. aureus*, *E. coli*) present in raw materials such as, fish, raw meats, and poultry may spread to other food items, such as cooked or raw foods, during food storage or preparation. Additionally, food products may become contaminated during food processing. A particular bacterial strain, temperature, pH, nutrient content, type of contact surfaces, and quality of contact surfaces are a few of the variables that might affect the formation and distribution of the biofilm. An effective approach is required, starting with the manufacturer guaranteeing a secure procedure and product design and anticipating potential issues. Fruit and vegetables are the important raw food consumed all over the world. Following China, India is the second-largest producer of fruits and vegetables. However, due to the losses in the field and during storage, their supply becomes insufficient. Roughly 30% of fruits and vegetables are spoiled after being harvested and become unfit for human consumption. According to estimates, soft rot caused by bacteria is responsible for 36% of vegetable degradation. Therefore, it is essential to implement appropriate measures for the safety of food.

Conclusions and Future Recommendations

Microbes that cause spoilage incur enormous losses in agricultural and food production impacting the national budget and pose threat to food security. To decrease the prevalence of foodborne infections, it is crucial for developing country governments, politicians, researchers, and the general public to work together. In poor nations, the use of quick procedures for detecting foodborne pathogens is necessary. To reduce harmful health impacts, proper safety measures must be taken during cooking and maintaining personal hygiene. Research must advance in the field of biofilm study methodologies to better comprehend and manage biofilms in food processing facilities. Getting rid of dead bacteria from food contact materials and preventing the initial microbial adhesion are challenges for the food business because the pathogenic bacterial biofilms are responsible for the spread of foodborne illnesses. Several emerging technologies have been identified as having the potential to increase the efficacy of materials used in food contact surfaces by reducing microbial contamination. Surface functionalization, high-intensity ultrasound, and cold plasma are surface decontamination approaches that are used to completely eradicate pathogens from food contact surfaces.

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Chapter 4
Application of Nanoparticles to Enhance the Microbial Quality and Shelf Life of Food Products

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Introduction

Raw and processed food products help people to obtain their intended energy, but raw products begin to exhibit reactions to deterioration after harvest and caused great waste all over the world. Food preservation is one of the widely used to address food waste concerns. The objective of food preservation is to block metabolic processes and prohibit the growth of bacteria or fungus (Saravanan et al., 2021). Due to relatively recent changes in the lifestyle of consumers, there has been a concomitant increase in the demand for products that are ready-to-consume or minimally processed (Białkowska et al., 2020).

Consumers require microbially safe, fresh or fresh-tasting, nutritious, shelf-stable, and accessible products made using ecologically friendly technology. Inadequately performed food processing procedures such as peeling, slicing, or washing, can present a danger to public health, particularly if these products are

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handled and disseminated inappropriately. In addition, microbial contamination with pathogen microorganisms can cause illnesses and lowered nutrition content in foods. Consequently, the effective control of spoilages established by bacteria is one of the most critical aspects of food production, processing, transit, and storage (Jaiswal et al., 2019).

Nanotechnology is the technique used to manipulate nanoparticles for a variety of applications. It plays a critical role in food and agricultural industries by promoting plant growth, enhancing food security and quality, and improving public health in novel and inventive ways (Duncan, 2011a). In addition, nanoparticles are used as nano additives, nanocapsules, gelating compounds, anti-caking factors, etc. in the food industry. Food safety, conservation, and functionalization are thus the core functions of nanotechnology in food security (Ghosh et al., 2020).

Nanoparticles (NPs) are tiny substances ranging in size between 1 to 100 nm. They are non-soluble or bio-persistent by nature, may be manufactured by a variety of methods, and are used in a variety of fields of study, such as the medical, electrical, agricultural, and food industries (Duncan, 2011b). Due to their prospective antimicrobial properties, silver (Ag), gold (Au), zinc oxide (ZnO), titanium dioxide (TiO_2) and carbon NPs are produced tenfold more than other nanomaterials. These NPs are used in air purifiers, food packages, deodorants, band aids, toothpastes, acrylics, and other consumer products (Kumari et al., 2009). In addition, the strong antimicrobial activity of nanosized copper oxides (CuO) has led to their widespread use in commercial nano-biocides (Nair et al., 2010). Food nanotechnologies employ nanoparticles of different sizes to provide healthier, safer, and high - quality products. Nanotechnology in food product packaging has demonstrated significant potential for enhancing the material characteristics of packaging. Nanocomposite antibacterial packaged techniques are effective, and large surfaces and the elevated surface energy of nanofillers produce positive interfacial interactions among polymer bonds and NPs. As a result, NPs substantially enhance biopolymer qualities such as thermal, mechanical, barrier and antibacterial activities (Sharma et al., 2020). This chapter explores the potential uses and applications of various NPs in food processing, microbial quality, storage time, as well as the ways in which NPs can be effective in extending the storage life and improving the quality of various products.

Food-System Nanoparticles

Inorganic NPs

Various types of NPs that have been used in food items are mostly constituted of inorganic components such as Ag, iron oxide, TiO_2, silicon dioxide, and ZnO. These components can be crystalline or amorphous, spherical or non-spherical at ambient temperature, exhibit variable surface features, are dependent on the original

materials and processing techniques for production and come in a range of sizes (Ghosh et al., 2019). Inorganic NPs, such as metallic NPs and nanoclays, attach to the pathogen cell membrane and cause inactivation by producing reactive oxygen compounds. Protein denaturation, DNA damage, and ion release may be attributable to reactive oxygen species (ROS) (Hoseinnejad et al., 2018).

Organic NPs

Organic NPs with antimicrobial capabilities include chitin nano-fibrils, nanofiber of cellulose or nanocrystals, and additional nanostructures generated from biopolymers using a solution casting technique to make carrageenan-based nanocomposites fortified with chitin nano-fibrils. Strong antibacterial action against *Listeria monocytogenes* was shown by the produced films. Additionally, grape seed extract is widely recognized for its significant antibacterial activity against Gram-positive (G+) microorganisms and has been used to generate antimicrobial biopolymer-based nanocomposite films for utilization in food packaging. (Jaiswal et al., 2019). Organic compounds may dissolve, accumulate, or be broken down in the mouth, stomach, small intestine or colon based on the composition and structures of the compounds. Organic NPs are assumed to be less hazardous than inorganic ones because they are broken down in the gastrointestinal tract (GIT) and are not bio-resistant (Ghosh et al., 2019).

Nanotechnology in Food Processing

Food processing refers to the way of preserving food using techniques to change the food into a state that is suitable for consumption. The main goals in food processing are to maintain the structure of the food and increase its storage period. The term nano-food refers to nanotechnology-processed, manufactured, secured, and packaged food. Nutritional supplementation, gelation and viscosification, nutrient delivery, vitamin supplements, and flavor nano-encapsulation are examples of nanomaterial-based food processing methods (Hossain et al., 2021). As gas and moisture barriers, edible nano-coatings (thin coatings around 5 nm) may be feasible for meat products, fruits, vegetables, cheese, processed food, and baked products. Nano-filters are applied to beetroot juice for discoloration without any damage to product flavor, and also, for production of lactose free milk by replacing lactose with other polysaccharides and making the milk appropriate for lactose-intolerant consumers (Bratovcic, 2020).

Advancement of NPs, or nano-textures, in food products as preservative factor or in packaging are subgenres of the use of nanotechnology in food processing. In general, nano-emulsions, surfactant microcapsules, emulsion multilayers, double or

multiple emulsions, and reverse micelles are the techniques employed to produce nanostructured food items. Examples of nano-textured food include spreads, mayonnaise, cream, yogurts, and ice creams, etc. (Jayakar et al.). Nanotechnology is effective in a term of food-borne illnesses prevention and production of healthier foods with less fat, sugar, and salt content. It has been reported that TiO_2 is approved as an additive in gums, sauces, and baked products (Weir et al., 2012). Furthermore, CuO, ZnO and iron oxide, have been classified as GRAS (generally recognized as safe) components for animal and plant products by the European Food Safety Authority (EFSA). Nutralease (enhancing nanoparticles to transport nutraceuticals and pharmaceuticals), Neosino capsules (nutritional supplements) and nano green tea are the most prevalent developed nanotechnology-based items currently on the market (He et al., 2019; Paidari & Ibrahim, 2021).

Nanotechnology for Food Preservation

Due to their antibacterial and physiochemical capabilities, usage of NPs is a popular method against pathogenic bacteria in healthcare, crop protection, water purification, food safety and preservation (Baranwal et al., 2018). The processing and preservation aspects of nanoparticles include nano-encapsulation, nano-emulsions, nano-formulations, and noncomposites. These innovative and novel usages of nanotechnology have the ability to solve a variety of issues faced by food technologists. For example, food NPs have active capabilities that may minimize a variety of food supply problems. Because of the obvious advantages of nanotechnology, its applications in food have grown dramatically (Donsì et al., 2011). The promising achievements of nanotechnology may be attributed to its low pollution, efficiency of energy, and small space needs. In addition, nanotechnology has several uses in agricultural, food, and environmental safety, toxicity, and risk assessment. (Anvar et al., 2019; Kaphle et al., 2018; Paidari & Ahari, 2021).

Nano-antimicrobials may prevent food deterioration and extend storage life. Many metal and metal oxide NPs have been suggested as antibacterial agents. Also ROS produced by attachments of metallic NPs to the pathogen cell membrane, have unique physicochemical properties, and caused oxidative stress and cell damage (Prasad et al., 2016).

Yu et al. produced silica in-situ poly-vinyl alcohol (PVA)/chitosan (CS) based films that are organic, cost-effective and possess very beneficial mechanical properties. These films decreased moisture and oxygen permeability by 10.2% and 25.6%, respectively, and tripled the cherry preservation period compared to standard packaging.(Yu et al., 2018). Chitosan NPs (CSNPs) have outstanding bioactivity and physiochemical properties that contribute to their growing popularity in food preservation (Yang et al., 2010).

Nanotechnology in Food Packaging

Food packaging techniques are designed to ensure that food quality is maintained and the food is safe for consumption. The packaging provides physical protection from external shocks and vibrations, microbiological contamination and heat by absorbing oxygen and other gases that contribute to product deterioration (Dera & Teseme, 2020; Esmaeili et al., 2021, 2022).

Most of the NPs used in food packaging have potential antibacterial action and prevent microbial spoilage. Through the controlled release of antimicrobials from the packed substance, packaging material composed of a coating of starch colloids containing the antimicrobial agent works as a barrier against microorganisms. NPs are employed as incorporate enzymes, antioxidants,, flavors and anti-browning agents carrier and other bioactive substances to extend the storage time of opened packages (Nile et al., 2020) (Fig. 4.1).

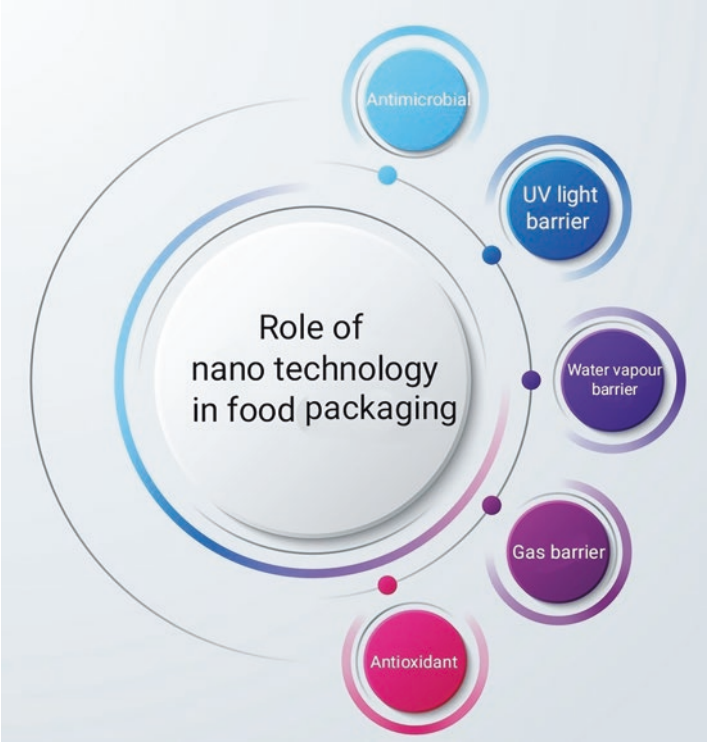

Fig. 4.1 Nanotechnology role in food packaging

Edible Film Packaging

Increased consumer demand for naturally preserved foods has prompted the food industry to investigate alternative preservation techniques. For example, edible biopolymers from renewable resources or industrial wastes are used in various packaging techniques (Hassan et al., 2018). Due to their benefits over synthetic films and their potential in preserving food, edible coatings have attracted great attention (Galus & Kadzińska, 2015). Nanotechnology has improved the functionality of edible films for food applications in recent years. It is feasible to include functional agents such as antimicrobials, anti-browning agents, antioxidants, enzymes, flavors, and colors by incorporating charged lipid or colloidal particles into nano laminated edible films (Duran & Marcato, 2013). Emamifar and Bavaisi revealed that nano-ZnO in edible sodium alginate coatings increased antioxidant activity and decrease microbial load, vitamin C content and weight loss. After 20 days, untreated strawberries exhibited greater antioxidant activity and phenolic degradation than coated ones (Emamifar & Bavaisi, 2020).

In another study, biodegradable starch-pectin-TiO_2-NPs composite edible films were created from sweet potato starch and lemon peel pectin containing TiO2-NPs. A small concentration of TiO_2-NPs improved the mechanical and moisture barrier properties of starch-pectin coatings, making them appropriate for food-grade biodegradable packaging material with UV protection (Dash et al., 2019).

Wang et al. created a Cheddar cheese-preserving edible film incorporating whey protein isolate nanofibers and carvacrol. Results show that the edible films have antibacterial action against, *Salmonella enteritidis,* Staphylococcus. *aureus, Escherichia coli,* and *L. monocytogenes*. Additionally, the antioxidant capacity was increased. Consequently, the authors asserted that whey protein nanofibers coatings might enhance the attributes of fresh cheddar cheese during storage (Wang et al., 2019).

Soltanizadeh and Goli examined the effect of aloe vera and eugenol bio-nanocoating on the physical and chemical parameters of fried shrimp. The coating significantly reduced quality loss during cooking and absorption of oil through frying and slowed the oxidation process during the freeze storage of samples. However, higher eugenol concentrations resulted indetrimental effects on the texture attributes of fried samples, and the combination of 2% aloe vera and 3% eugenol nanoemulsion proved to be the best coating material (Sharifimehr et al., 2019).

Tabari evaluated the impact of adding carboxymethyl cellulose (CMC) NPs to sago starch on water absorption capacity, physiochemical characteristics density, and sealability of films. By increasing CMC NPs concentration, mechanical characteristics increased significantly ($P \leq 0.05$) and tensile strength and elongation parameter considerably ($P \leq 0.05$) decreased from 17.69 to 15.39. Consequently, this film may be utilized as an edible film for food products and pharmaceutical packaging in different sectors, notably the food industry (Tabari, 2018).

Using ZnO nanorods (ZnO-nr), Jafarzadeh et al. fabricated nanocomposite films by solvent casting. The ZnO-nr particles were uniformly dispersed across the film

surface, as demonstrated by SEM pictures. The amount of ZnO-nr significantly affected semolina coating absorbance and adding ZnO-nr considerably decreased oxygen permeability and thermal sealability, as revealed by the results. The nanocomposite coatings absorbed more than 90% of the near infrared spectrum (Jafarzadeh et al., 2017). The edible films of whey proteins incorporating TiO_2 NPs displayed superior physiochemical, moisture barrier, and antimicrobial characteristics (Sani et al., 2017).

Nano-encapsulation

Encapsulation includes wrapping bioactive molecules with a barrier component or enclosing them inside shells or carriers.

Encapsulation's primary purpose is to shield the vital ingredient from damaging external factors such as light, humidity, and oxygen. This will extend the product shelf life and provide controlled encapsulation extraction, In addition, ease of handling, enhanced stability, prevention of oxidative stress, preservation of volatile substances, taste modification, moisture-triggered controlled release, pH-triggered controlled release, active compounds continuous delivery, flavor character change, long-lasting organoleptic impression, and improved bio-accessibility and effectiveness are other applications of encapsulation (Pradhan et al., 2015).

The small size of the bioactives inside the capsules facilitates their distribution to the intended location. Nanocapsules are preferable to microcapsules in terms of stability, solubility, and encapsulating efficiency (Malik et al., 2019). Various encapsulating processes, such as nanoemulsion, coacervation, the extrusion technique, fluidized bed coatings, spray chilling, and spray drying, have been utilized to create nano or micro-particle systems (Bajpai et al., 2018).

Most of the bioactive molecules such as lipids, proteins, polysaccharides, and minerals are susceptible to the high acidity and enzyme activity of the mucosal lining of the GI tract. Encapsulation of these bioactive components promotes their absorption in food items, which is difficult to do in their un-encapsulated state due to their low water solubility (Singh et al., 2017).

Based on wall material chemical structures that are used for nano-encapsulation of food components, nanostructures are divided into three types: (1) lipid-base nanosystems, such as archeosomes, solid lipid NPs, colloidosomes, nanocochleates, and nanoliposomes; (2) polymeric-type nanosystems, such as, carbohydrate-based NPs, pectin, chitosan nanofibers, cellulose, dextran, guar gum, alginate, and starch; and (3) protein-base nanosystems, such as zein ultrafine fibers, milk protein nanotubes and corn protein (Ghosh et al., 2019). Liposome is an indication of a nanotransporter that is utilized for nano-encapsulation of various components. Nano-transporters are applied for carrying nutraceuticals, minerals, proteins, vitamins, antimicrobial compounds, and additives. Due to the superior solubility and specificity of the encapsulated elements, lipid-based encapsulation techniques are more effective than other alternative encapsulation systems (Dera & Teseme, 2020).

Carbohydrate NPs used for oil encapsulation are digestible or indigestible polysaccharides such as sodium, alginate, pectin, and cellulose. Physicochemical stability and solubility of algal oil NPs demonstrate a system efficiency of 98.57 (Wang et al., 2020).

Mohammadi et al. investigated the influence of a CSNPs coating containing *Zataria multiflora* EO on the storage life and antioxidant activity of cucumber. In this study CSNPs-*Z. multiflora* composites with a weight ratio of 1:0.25 were prepared and had an acceptable encapsulation efficiency and load capacity. After 21 days of storage at 10 ± 1 °C, cucumbers with CSNPs -*Z. multiflora* composites exhibited superior quality compared to those with simply CSNPs coating or distilled water treatment (Control) (Mohammadi et al., 2016). Lipids, β-carotene, nisin, coenzyme Q10, oregano essential oil (EO), and particular probiotics are nano-encapsulated nowadays. Release time of encapsulated EO delayed and extended by utilizing CS/cashew gum. Encapsulated samples exhibited potential bactericidal effect against *Stegomyia aegypti* larvae (Abreu et al., 2012; Esmaeili et al., 2021, 2022). Imran et al. created soy and marine lecithin-based liposomal nano-delivery technologies for encapsulating the food preservative nisin (Imran et al., 2015). In another study, 1-octenyl succinic anhydride refined starch (OSA-ST) was used for coenzyme Q10 encapsulation. Coenzyme Q10 dissolved in rice bran oil and added into OSA-ST solution. Results indicated that the dietary supplement coenzyme Q10 had been effectively nano-encapsulated with this combination and particle size of this mixture was about 200–300 nm (Cheuk et al., 2015). Zhang et al. revealed that compared to non-capsulated thymol EO, thymol encapsulated inside a zein NPs successfully inhibited the development of G+ pathogens (Zhang et al., 2014).

Nanoemulsion

Nanoemulsions are applied in the manufacturing of salad dressings, sweeteners, flavored oils, customized drinks, and other processed products. Using a variety of inputs such as heat, pH, ultrasound waves, etc., nanoemulsions assist in the release of various flavors. They effectively preserve the sensorial attributes and protect them from oxidation and enzyme processes. Compared to traditional emulsions, nanoemulsions serve as exceptional carriers for many bioactive substances by providing premier features such as high optical clarity, physical properties such as texture and aggregation, and increased bioavailability (McClements & Rao, 2011).

Nano-emulsions are oil-in-water emulsions and range in size from 50 to 200. The nano-droplet size provides nanoemulsions with remarkable transparency. When added to food, nanoemulsions display rheological and textural properties that are highly stable through extended time periods (Malik et al., 2019). Various food-borne pathogens, such as Gram-negative (G-) bacteria, are strongly inhibited by nanoemulsions (Nile et al., 2020).

Nano-emulsions are produced by distributing liquid phase in water phase in a continuous process. The components utilized to create nano-emulsion are

lipophilic, with the lipophilic component being extensively incorporated into the oil phase. Numerous factors, including the molecular and physicochemical features of the component, dictate the position of the lipophilic component inside the nano-emulsion. Nanoemulsions have the physical attributes of hydrophobicity, surface activities, oil-water diffusion index, dispersion, and melting temperature. By creating nanoemulsions, several lipophilic substances are encapsulated. Nano-emulsions are preferred over conventional emulsions due to their smaller droplets, larger surface area, and faster digestion and absorption by digestive enzymes (Pradhan et al., 2015).

Due to their activity, and non-toxicity, nano-emulsions in edible coatings have recently attracted significant attention. However, the nano-system is not well commercialized. Nano-emulsions in edible coatings include flavor, coloring, antioxidant agents, and antimicrobials for meats, dairy, and fruits application (Aswathanarayan & Vittal, 2019). As antimicrobial agents, nano-emulsion based oregano EO was added to low-fat sliced cheese in order to improve its storage period (Artiga-Artigas et al., 2017).

Antimicrobial Properties of Nanoparticles

Pathogens and antimicrobial-resistant organisms that cause spoilage in food products are a significant public health concern; thus, several studies have been conducted with the purpose of enhancing recent antimicrobial methods. Metal NPs such as Ag, Zn, Cu, gold (Au), and titanium (Ti) exhibit diverse antibacterial activity features, with potencies and activity spectra that have been recognized and used for decades (Malarkodi et al., 2014). The antibacterial properties and efficiency of NPs are significantly impacted by the composition and their droplet size (Khezerlou et al., 2018) (Table 4.1).

Recently, Muhammad Bilal Khan Niazi et al. produced biodegradable nanocomposites for antibacterial application in food packaging. Both G+ and G- organisms were susceptible to the antibacterial capabilities of the coatings, and a powerful antibacterial effect was found against G- bacteria (*Escherichia coli (DH5-alpha)*) (Sarwar et al., 2018).

CSNPs were created using ion gelation for the fabrication of starch-based nanocomposites. The antibacterial properties of starch/CSNPs films were investigated by a disc diffusion study in vitro and microbial count in film-wrapped cherry tomatoes in vivo experiments. For all bacteria studied, including *Bacillus cereus, Staphylococcus aureus, E. coli,* and *Salmonella typhimurium*, the inhibitory zone of starch/CSNPs films (15% and 20% w/w) was identified (Shapi'i et al., 2020).

Ag was the most common inorganic antimicrobial NP (Zinjarde, 2012). Various plastic and biodegradable coatings benefit greatly from the antibacterial impact of Ag. In addition, Ag NPs have several biological uses (Khezerlou et al., 2018) (Fig. 4.2).

Table 4.1 Bio-nanocomposite coatings as packaging to increase the microbiological stability of food products

Bio-nanocomposite	Food product	Results	References
Semolina/ ZnO- NPs & nanokaolin	Low-moisture Mozzarella	The bio-nanocomposite coatings maintained the physical and sensory qualities of cheese and inhibited microbiological activity for 72 days.	Jafarzadeh et al. (2019)
Gelatin/cellulose nanofibrils/ag NPs	Fruits and vegetables	Appeared significantly effective against *E. coli* and *S. aureus.*	Li et al. (2019a, b)
Pectin/nanohybrid-layered double-hydroxide salicylate	Apricots	Enhanced pectin elongation at breakpoint; enhanced water vapor barrier characteristics; extended the storage period.	Gorrasi and Bugatti (2016)
PLA/PBAT/nanocrystal cellulose-silver nanohybrids	–	Antimicrobial properties were exhibited against *E. coli* and *S. aureus*.	Ma et al. (2016)
Chitosan and mandarin EO Nano-emulsion	Green beans	Decrease in the *L. monocytogenes* population	Donsì et al. (2015)
Chitosan–silver nanocomposite	–	Antimicrobial properties against *L. monocytogenes, E. coli, S. aureus,* and *S. typhimurium*.	Rhim et al. (2013)
Pullulan films/NP (silver or ZnO NPs)/ oregano or rosemary EOs	Turkey deli meat	The antibacterial impact of pullulan nanocomposites was retained at low temperatures (< 25 °C) but was drastically diminished at temperatures over >25 °C.	Khalaf et al. (2013)
PLA and AgNO3	Fresh-cut vegetables	Exhibited strong antifungal and antibacterial activities with increasing Ag concentration.	Martínez-Abad et al. (2013)
Cellulose absorber/Cu	Melon and pineapple juices	Strong antifungal action, decreasing yeasts and molds related to deterioration.	Llorens et al. (2012)
Sodium alginic acid silver-montmorillonite nanoparticles	Fiordilatte cheese	Promoted microbiological stability by inhibiting *Pseudomonas* spp. growth.	Gammariello et al. (2011)
Silver NPs immobilized in cellulose and collagen	Sausage	Antibacterial efficacy against *E. coli* and *S.aureus*	Fedotova et al. (2010)

Recent investigations have shown that Ag NPs are safe for application for packaging and films in the food industry with no measurable or negligible quantities of migration from saturated containers into food samples and food simulants. Nanocomposites provide high stability, which is crucial for preserving antibacterial action and decreasing the chance of metal ion migration into preserved foods (Aziz et al., 2019).

Several researchers have reported the green production of Ag NPs from silver salts utilizing parsley (*Petroselinum crispum*) leaf extract, celery leaf extract, etc. Apple and cucumber extraction are effective for the production of Ag NPs (10 nm)

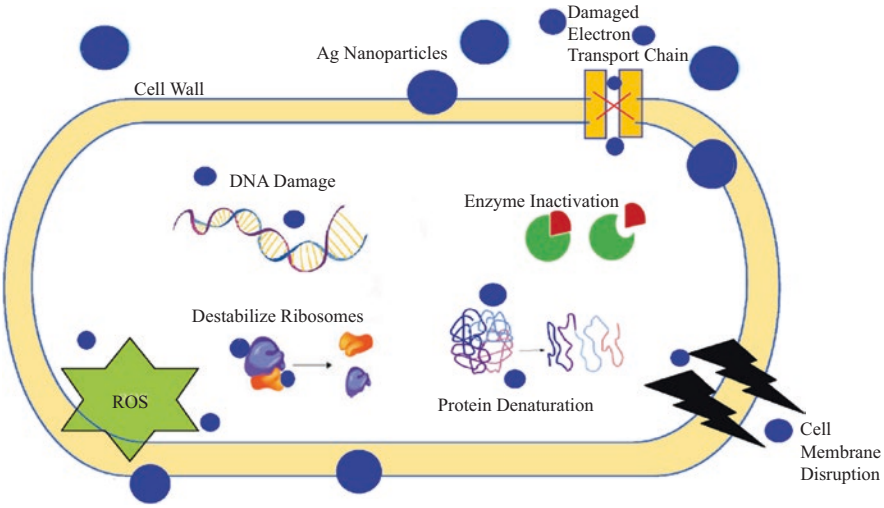

Fig. 4.2 Antimicrobial mechanism of Ag NPs

among fruit extracts. The effect of PLA nanocomposites containing bergamot EO, TiO_2 and Ag NPs on the storage time of mango samples at ambient temperature for 15 days was evaluated by Chi et al. Conclusions proved that PLA/NPs films could preserve the freshness of mango, extend its storage life up to 15 days and delay the weight loss of samples (Chi et al., 2019).

Ag NPs were also tested against *S. typhimurium, Listeria monocytogenes, Vibrio parahaemolyticus,* and *E. coli*. According to the results, Ag NPs exhibited significant antimicrobial impacts. Thus, Ag NPs are a viable option for disinfecting surfaces and equipment that come into contact with food products (Khezerlou et al., 2018).

Shahrokh and Emtiazi discovered that Ag NPs at small concentrations (0.2 ppm) stimulated bacterial activity. As a result, the researchers proposed using an optimal concentration of Ag NPs for different nanomaterials in order to minimize biofilm development (Shahrokh & Emtiazi, 2009).

ZnO is a safe supplement for food applications and coatings that have direct contact with food and the human skin and body (Ravindranadh & Mary, 2013). In fact, ZnO NPs have antibacterial effect against both G+ and G- bacteria and are high pressure and high temperature resistant (Khezerlou et al., 2018). Compared to micro-particles, ZnO NPs have excellent antibacterial activity due to their surface area (Seil & Webster, 2012). According to Emamifar and Bavaisi, inserting nano-ZnO in edible sodium alginate coatings boosted its antioxidant activity anddecreased the quantity of oxygen needed for the oxidative stress of anthocyanin and phenolic compounds (Emamifar & Bavaisi, 2020).

The antibacterial impacts of ZnO NPs against pathogens and spoilage microorganisms in food products was investigated by Espitia et al. ZnO NPs exhibited no

significant antibacterial effect *against Pseudomonas aeruginosa, Lactobacillus plantarum,* or *L. monocytogenes* but showed substantial antibacterial action against *Saccharomyces cerevisiae, Salmonella choleraesuis, S. aureus, E. coli,* and *Aspergillus niger* (Espitia et al., 2013).

Due to their nontoxicity, polyvalent impacts, high capacity to be functionalized, detection efficiency and photothermal activities, gold (Au NPs) are regarded as useful in the creation of antibacterial agents (Lima et al., 2013; Lokina & Narayanan, 2013). Researchers have developed an EO droplet emulsified with gold NPs and have also used NPs to encapsulate peppermint and cinnamaldehyde EOs (Schmitt et al., 2016).

Based on the findings, Au NPs reduced *Salmonella typhi* and *E. coli* colonies by 90–95%. According to the researchers, the distribution and hardness of Au NPs on the medium were the primary parameters influencing the bactericidal characteristics (Lima et al., 2013).

Clay NPs are composed of mineral silicate layers. Based on their chemical composition and form, these NPs are categorized into montmorillonite(MMT), hectorite, kaolinite, bentonite, and hydroxyapatite (Mierzwa et al., 2013). In nanomaterials applications, MMT is the nanoclay that is utilized most often. Depending on the surface modification of the clay layers, MMT may be dispersed in a polymeric combination in order to produce a nanocomposite, (Shameli et al., 2011).

Paulraj Kanmani et al. determined the antimicrobial properties of gelatin, Ag NPs, and nanoclay bioactive nanocomposites against *E. coli* and *L. monocytogenes*. Ag NPs revealed significant antimicrobial properties against both pathogens, but clay NPs were only effective against G+ (*L. monocytogenes*) pathogens (Kanmani & Rhim, 2014). In another study, the antibacterial impact of Cu NPs/ MMT clay was examined. The composition exhibited excellent antimicrobial effects against *E. coli, S. aureus, Pseudomonas aeruginosa,* and *Enterococcus faecalis*. (Bagchi et al., 2013).

Application of NPs in Food Products

Nanotechnology in Fruits and Vegetables Preservation

Fruits and vegetables are at the top of most shoppers' lists, particularly with regard to vitamin, mineral, antioxidant, and fiber content. However, due to their high water content (about 75–95%), fruits and vegetables face a limited storage life with consequent rapid degradation and an unattractive appearance during storage (Otoni et al., 2017). Nevertheless, it is possible to extend the storage time for fruits and vegetables by using proper packaging techniques. Nanocomposite antimicrobial packaging technologies are ideal options to address storage time considerations through enhanced mechanical, barrier, heat, and antibacterial qualities (Jafarzadeh et al., 2019) (Table 4.2).

Table 4.2 Application of nanoparticles for fruits and vegetables preservation

Fruits or vegetables	Nano-components	Advantages	References
Cut papaya	Chitosan/ZnO	The coating dramatically reduced microbial growth in fresh-cut papaya during storage.	Lavinia et al. (2019)
Banana	Isolate soy protein (ISP)/ ZnO NP	Nanocomposite film may delay banana ripening and loss of weight, titratable acidity, prevent changes in sensory attributes, total soluble sugar, fungal growth and firmness during storage.	Li et al. (2019a)
Black grape	Phthalate/ cellulose acetate// chitosan ZnO NPs	The storage life of black grapes by use of ZnO NPs increased up to 9 days.	Indumathi et al. (2019)
Vegetables and fruits	Chitosan/ZnO/ Melissa EO	Improved antimicrobial properties of chitosan coating.	Sani et al. (2019)
Orange fruit	Carnauba wax/ Clay NPs	Significantly improved the sensory acceptance of oranges, nutrition value, loss of weight, and respiration rate.	Motamedi et al. (2018)
Carrot	ZnO NPs	ZnO NPs may lower the overall number of colony-forming units (CFU) during storage, thus extending the storage period up to 40 days.	Xu et al. (2017)
Pomegranate	ZnO NPs	Nano coating reduced yeast and mold and weight loss.	Saba and Amini (2017)
Strawberries	ZnO NPs	ZnO NPs prevented microbial growth, delayed weight loss, and preserved strawberry nutrients.	Sogvar et al. (2016)
Cantaloupes	Chitosan/Ag/TiO_2	Prevents microorganism development and has strong antibacterial action against target microorganisms.	Lin et al. (2015)

Fruits can be categorized as climacteric or non- climacteric where fruits that can ripen after harvesting are climacteric and those that cannot ripen after harvesting are non- climacteric (Farcuh et al., 2018). As fruits and vegetables are still living tissues after harvesting, they have a limited storage period and can degrade rapidly during storage and transit due to chemical reactions, physiological aging, and microbiological infections. Consequently, there is often a decrease in the edibility of these products, resulting in a considerable annual loss of fresh fruits.

Due to the absence of effective shelf-life extension techniques, about 20–40% of fruits and vegetables spoil and deteriorate annually. Furthemore, by appropriate preservation method, ripening of fruits and vegetables delayed and the shelf life extend, microbiological contamination limited and transpiration of products as a freshness-maintenance strategy facilitated. Researchers have suggested several methods such as MAP packaging, waxing, and biodegradable composites for preserving vegetables and fruits. Among these methods, bio-nanocomposites or edible

coatings are well recognized for their ability to preserve the postharvest quality of fresh fruits and vegetables (Jafarzadeh et al., 2021).

The impacts of isolate soybean protein (ISP) film and ISP/CIN/ZnO NPs composite coating on the postharvest quality of bananas during their storage time were investigated. The bio-nanocomposite covering was shown to maintain positive banana attributes by delaying ripening and reducing oxygen transport in samples (Li et al., 2019a).

Lavinia et al. demonstrate the application of CS and ZnO NPs as a novel coating on fresh papaya. Results have shown that the incorporation of nanocomposite coating in samples may significantly limit microbial activity during storage compared to uncoated samples and nanocomposite treatment may provide an alternate technique for the preservation of freshly sliced papaya after harvest and process (Lavinia et al., 2019). Chi et al. demonstrated that the polylactide (PLA) nanocomposite containing Ag NPs and TiO_2 NPs may effectively prevent the loss of mango firmness during storage. In addition, as compared to PLA films, nanocomposite films have the potential to limit vitamin C degradation, color and total acidity changes, and inhibition of microbial load in mangos (Chi et al., 2019).

Ethylene generation and respiration rates among fruits and vegetables can vary. During the ripening stage, fruits release ethylene and increase their respiration rate. Non-climacteric fruits generate a small quantity of ethylene and do not react to treatment with ethylene (Tripathi et al., 2016). Cherries, grapes, lemons, oranges, blueberries, raspberries, cucumbers, pomegranates, and watermelons, which are non-climacteric, must remain on the tree until they reach complete physiological ripening. Once harvested, these fruits will no longer continue to ripen, produce sugar, or acquire taste. Researchers have suggested the use of edible coatings and nanocomposite films to increase the shelf life of these fruits, which are very important due to their nutritional value, unique sensory attributes, and bioactive components (Chen et al., 2018). According to Fadeyibi et al., cassava starch bio-nanocomposites modified with ZnO NPs enhanced the storage period of cucumbers (Fadeyibi et al., 2020).

Strawberry was coated with a nano-biodegradable coating composed of sodium alginate and ZnO NPs generated by Emamifar et al. ZnO NPs significantly improved the water resistance of the coatings and, as a consequence, decreased strawberry weight loss. At the conclusion of storage (20 days), the uncoated fruits show higher weight loss in comparison with coated fruits with nano-biodegradable coating (Emamifar & Bavaisi, 2020).

Salama et al. used aloe vera gel, alginate, and TiO_2 NPs for bio-nanocomposite coatings preparation for extending the shelf life of tomatoes and an edible film based on carboxymethyl cellulose (CMC) for green bell pepper smart packaging. The results demonstrated that edible films significantly postponed spoilage and weight loss in tomatoes (Salama & Aziz, 2020). Sarojini and Rajarajeswari produced a biodegradable cellulose acetate phthalate/CS coating that was applied to black grapes and had varying percentages of ZnO NPs. The coatings containing ZnO NPs (5%) increased the storage period of black grapes up to 9 days (Indumathi et al., 2019).

Kumar et al. created bio-nanocomposite coatings using agar and ZnO NPs for green grapes. The findings showed that green grapes remained fresh up to 21 days vs. films containing 4% ZnO NPs (Kumar et al., 2019). Kaewklin et al. demonstrated the use of active packaging including CS/TiO_2 NPs for the preservation of tomatoes at 20 °C. The packed tomatoes exhibited less deterioration than the control film samples. Additionally, coatings containing NPs delayed the maturation process of tomatoes (Kaewklin et al., 2018).

Nanotechnology in Cheese Preservation

Cheeses are particularly sensitive to surface contamination by microorganisms due to their favorable acidity and high water content (Proulx et al., 2017). The majority of studies on cheese storage and shelf life have focused on concerns related to contamination caused by microorganisms. In addition, the increased moisture loss in certain cheeses due to the lack of a packing barrier may increase product hardness and lead to undesirable organoleptic qualities (Mei et al., 2020). Nano-systems are potential antimicrobial agents in the food industry, and different studies have examined the effect of bio-nanocomposites on a variety of cheese products, focusing on NPs (Resa et al., 2016).

El-Sayed et al. examined the effect of CS/guar gum/Roselle calyx extract (RE)-ZnO bio-nanocomposites for coating of Ras cheese. In addition, the physiochemical, microbial, and sensory aspects of Ras cheese among ripening in comparison to uncoated cheese were examined. Coated samples with a bio-nanocomposite layer comprising 3% RE-ZnO NPs exhibited significant effects against yeasts, molds, and other microorganism growth for approximately 3 months (El-Sayed et al., 2020).

Amjadi et al. determined the effectiveness of the gelatin-based nanocomposite comprising CS nanofiber (CSNF) and ZnO NPs for packing chicken fillets and cheese. The nanocomposite coating of samples considerably inhibited the development of inoculated bacteria ($p \leq 0.05$). Moreover, the sensory qualities of packed samples with CSNF and ZnO NPs were acceptably maintained during the storage time (Amjadi et al., 2019).

In another study, ecofriendly, cost-effective, and sustainable materials containing chitosan, PVA, glycerol, and TiO_2-NPs were created. Karish was manufactured, coated with a bio-nanocomposite comprising 1, 2, and 3% TiO2-NPs, and then refrigerated. The quality of covered Karish cheese was acceptably maintained until the end of the storage period; however, uncoated samples showed surface fungal growth and after 15 days, the quality of control sample was unacceptable. Karish cheese covered with a bio-nanocomposite containing 3% TiO_2-NPs was rated highest in terms of acceptance at the end of the storage period (Youssef et al., 2018).

Divsalar et al. used chitosan-cellulose, nisin, and ZnO NPs for packing ultra-filter white cheese and to increase its shelf life. Findings revealed that the nisin-containing bio-nanocomposite layer enhanced the storage time of ultra-filter white cheese and inhibited the development of microorganisms on the cheese surface layer for 14 days at 4 °C (Divsalar et al., 2018).

Nanotechnology in Seafood Preservation

Due to their low-calorie content, omega 3 fatty acid, vitamins, minerals, and protein content, aqua food products (AFPs) are often favored by customers. However, microorganisms, enzymes, and chemical processes quickly deteriorate AFPs. In response to changing consumer preferences with regard to safer food products, researchers have concentrated on using nanotechnology in AFPs preservation (Çiçek & Özoğul, 2022). The foundation of nanotechnology for the preservation of AFPs are NPs. The majority of applications for NPs have been employed to maintain AFPs. TiO_2 NPs contain strong antibacterial properties and can suppress aquatic pathogens in vitro (Noman et al., 2019).

Mehdizadeh et al. examined the efficiency of Cs-zein coating containing free and nano-encapsulated *Pulicaria gnaphalodes* (Vent.) *boiss* extract on quality attributes of rainbow trout stored at 4 °C for 14 days. By utilizing this coating, peroxide value and thiobarbituric acid decreased during storage (Mehdizadeh et al., 2021). The edible coating developed by Ag NPs, *Satureja rechingeri* extract, and PVA effectively inhibited growth of *S. aureus, E. coli*, psychrophilic bacteria and mesophiles on rainbow trout fillets (Kavakebi et al., 2021). Kargar et al. investigated the antimicrobial effects of Ag/Cu/ZnO NPs generated by chemical reduction technique in order to increase the shelf life of caviar during the storage period (14 days). Results indicated that the total amounts of volatile nitrogen and thiobarbituric acid decreased significantly (Ahari et al., 2021).

Maghami et al. studied the impact of CSNPs loaded with fennel EOs and the modified atmosphere packaging (MAP) technology on the biochemical, microbial, and sensory attributes of Huso huso fish fillets during storage. The findings demonstrated that coating fish fillets with CSNPs and fennel EO considerably decreased the peroxide value and thiobarbituric acid value in compared to the control samples, thereby extending the product storage life (Maghami et al., 2019).

Durmuş et al. studied the effect of nano-emulsions based on trading oils (hazelnut oil, corn oil, canola oil, soybean oil, olive oil, and sunflower oil) on vacuum-packed sea bass fillets. The shelf life of samples treated with nano-emulsion kept at 2 ± 2 °C was extended by approximately 2–4 days. Fish fillets treated with hazelnut and corn oil groups exhibited lowest bacterial growth and lactic acid bacteria. Results proved that the storage time of fish samples was extended up to 4 days with nano-emulsions of canola, corn, soybean, and hazelnut oils vs. only 2 days by emulsions of olive and sunflower oils (Durmus et al., 2019).

To preserve the silver carp fish ball, Wei et al. developed a composite CS film contain ZnO/ TiO_2 and SiOx (ZTS-CS). The textural change and freshness indicators of fish ball were extended as prepared films have significant antibacterial activities and the gas permeability of the films was appropriate. Moreover, the quality of fish ball coated with ZTS-CS was maintained for 24 days vs. about 5 days for the control samples (Wei et al., 2018).

Mizielinska et al. examined the firmness and microbial load of cod (*Gadus morhua*) fillets packaged with a methyl hydroxypropyl cellulose coating modified

with ZnO NPs. The coating reduced gumminess in the samples which significantly improved the texture quality. Mesophilic and psychotropic bacteria counts decreased in coated Baltic cod (***Gadus morhua***) at 5 °C for 144 h (Mizielińska et al., 2018).

Ramezani, et al. reported that due to the size effect, bulk CS coating is less effective than CSNPs at inhibiting microbial loads on fillets of silver carp *(Hypophthalmicthys molitrix*) stored at 4 °C for 12 days. The total psychrotrophic and mesophile bacteria counts remarkably decreased in samples coated with CSNPs (Ramezani et al., 2015). Budhijanto, Nugraheni, and Budhijanto discovered that CSNPs are more efficient than chitosan in antibacterial compounds when applied to fresh tilapia (*Oreochromis* sp.) at cold temperatures. Compared to samples preserved at ambient temperature (25 °C) and untreated with CSNPs, samples kept at low temperatures (10–15 °C) and covered with CSNPs had a substantial positive impact on preventing the development of microorganisms (Budhijanto et al., 2015).

Nanotechnology in Beverage Preservation

Nanotechnology is not novel to the food and beverage industry, as several novel nano-based approaches have already been used in functional and nutraceutical food applications, production, and processing. Utilizing colloid technology, food production may be enhanced over an extended preservation time. This is due to the utilization of nanoscale-sized ingredients in the majority of beverages and foods such as dairy products. Therefore, the decrease in size of these substances at the nanoscale range should be considered (Chaturvedi & Dave, 2020).

In many sauces, beverages, oils, and juices, NPs have shown a variety of electrochemical and visual characteristics. The incorporation of nano-emulsified bioactives and flavors to beverages has no impact on the appearance of the product (Rhim et al., 2013). A recent study demonstrated that CS nano-composite may also be employed for the clarifying, stabilization, and encapsulating of alcoholic, non-alcoholic, and dairy-based drinks, juices, teas, and coffees (Morin-Crini et al., 2019).

Toxicological, Safety, and Migration Issues of Metal NPs in Food Products

Toxicological Aspects of NPs

Nanotechnology science continues to expand, and along with this growth has come an increase in public health concerns regarding the toxicity and environmental effects of nanomaterials. In addition to functionalization, agglomeration, and net particle response, dynamic, kinematic, and enzymatic features- along with enzymatic activity- increase the toxicity of NPs (Zou et al., 2016). Toxicokinetic

problems generated by NPs are primarily attributable to their persisting insolubility and nondegradable features (López-Serrano et al., 2014). As the size of metal NPs decreases, their toxicity increases. NPs are highly reactive chemicals that easily penetrate membranes and capillaries, generating toxico-kinetic and toxico-dynamic effects (Hajipour et al., 2012).

NPs can enter the body by ingestion, skin contact or inhalation (Maisanaba et al., 2015). Once they enter the biological environment, NPs will inevitably interact with biomolecules in the bloodstream, such as proteins, carbohydrates, and lipids (Farhoodi, 2016). Some NPs link to enzymes and proteins which stimulates the generation of reactive oxygen species (ROS) and oxidative stress. ROS production induces mitochondrial degradation and cell death (Hajipour et al., 2012).

ZnO NPs exhibited genotoxicity in the human epidermis even though ZnO in bulk size is non-toxic, indicating the importance of particle size (Sharma et al., 2009).

Vishwakarma et al. evaluated effects of AgNPs and Ag nitrate on the growth of hydroponic mustard (*Brassica* spp.). Both chemicals affected the length of root, fresh weight, ascorbate peroxidase, total chlorophyll and carotenoid composition, protein content, catalase activity, oxidation, DNA degradation, compound aggregation, and plant cell growth (Vishwakarma et al., 2017). Echegoyen and Nern detected Ag migration in all three samples of industrial AgNPs plastic containers, with total Ag migration varying from 1.66 to 31.46 ng/cm^2 (Echegoyen & Nerín, 2013). To eliminate the challenges related with nanotechnology in the food sector, the bioavailability, behavior, and toxicity of NPs in the environment should be thoroughly investigated (Lugani et al., 2021).

Safety of Food Products

Food safety is a worldwide health concern, and food safety measures help to ensure that preparation and consumption will not harm the health of consumers (Pal, 2017). Recent developments in nanotechnology have changed the food industry with regard to food processing, security, and safety, in addition to advancements in improving nutraceutical content, prolonging storage time, and minimizing packaging waste (Wesley et al., 2014). Pathogens, pesticides, and other pollutants in food represent significant health risks to humans. Nanotechnology advancements have accelerated solutions to food safety challenges with microbiological contamination and enhanced toxin identification, and storage time (Inbaraj & Chen, 2016).

Another prospective application of nanotechnology is for detection of levels of toxic elements pathogens, and microbial load in food systems. The interesting new concept of combining biology and nanotechnology into sensors is promising, since the reaction time to detect a possible danger would be dramatically lowered. This will result in increased food processing system safety. A research program in Iowa State University by Launois revealed that Ag NPs might boost the safety of the worldwide food supply (Launois, 2008). Currently, Ag NPs cannot be directly added to food products due to a lack of research on their detrimental effects on

human health and ecological systems. In order to develop food-related applications such as microbe-resistant materials and non-biofouling surfaces, the research program examines how Ag NPs may operate as antimicrobial agents in meals (Alfadul & Elneshwy, 2010).

The U.S. Food and Drug Administration (FDA) is charged with securing human health by regulating the safety of substances that are directly in contact with food. For example, the FDA plays a significant role in testing the safety of NPs contained in food products and nanoscale food ingredients (Paradise, 2019). The FDA has published a number of nanotechnology-related reports and suggestions for research, analysis, and regulatory policy in order to assist the sector. Based on comments by the FDA Commissioner (Paradise, 2010), nanotechnology has been acknowledged for dealing with nanotechnology-based food products. Moreover, according to the standards of European nations, nanomaterials (100 nm or less) may only be used if they are allowed and mentioned in rules of Annex I, and their migration levels in food products must be below detectable limits.

Migration of Metal NPs into Food Products

Determining the optimum migration of NPs into food is one of the food industry's primary issues. Migration is defined as the mass transfer of particles with a low molecular weight (Zamindar et al., 2020).

The migration of heavy metals from nanomaterials is a cause for major concern (He et al., 2015). In the case of long-term agglomeration, the distribution of heavy metals into food items has negative consequences. For example, metal and metal oxide NPs, including Ag (McShan et al., 2014), and CuO (Karlsson et al., 2013), ZnO increase intracellular ROS levels, causing lipid oxidation and DNA damage (Fukui et al., 2012). Allergies and the release of heavy metals as the migration phenomenon are the two main safety concerns of NPs. AuNPs depict a good safety profile. Considering AuNPs, as well as other metal NPs have the potential of toxicity. AuNPs are capable of migration from packaging to food matrix and finally, they will be released in the human body after food consumption which is a toxin for different cells and tissues (Bindhu & Umadevi, 2014). In recent years, Simpson et al. made, analyzed, and validated carbon NPs (66 nm) with glycerol for detecting heavy metal ions with a 0.30 ppm detection limit (Simpson et al., 2018). Lingamdinne et al. demonstrated that NPs of iron oxide that are produced and reused without affecting stability may reduce heavy metals in products (Lingamdinne et al., 2017).

Amal M. Metak et al. studied AgNP migration from nanosilver sheets and juice packing. Nanosilver-coated films generate substantial migration levels (0.03 mg L^{-1}), and this is cause for concern regardless of whether the metal is in the form of NPs or ions. However, no chemical or biological alterations were detected in the food items analyzed (Metak et al., 2015).

In another investigation, a migration experiment was conducted in order to see how time and temperature affected the migration of CuNP/AgNP from polyethylene

nanocomposites to chicken breasts. According to the findings, neither time nor temperature had a major impact on migration. Migration of copper and silver varied between 0.024 and 0.049 mg/dm^2 and 0.003 and 0.005 mg/dm^2, respectively (Cushen et al., 2014).

Cushen et al. studied AgNP migration from nanocomposite PVC on chicken breasts, and findings revealed migration levels ranging from 0.03 to 8.4 mgkg^{-1} (Cushen et al., 2013).

Conclusion

Nanotechnology has revolutionized the food processing and preserving industry. It is an innovative technology that offers a promising route for developing novel packaging components. By mixing polymeric materials with organic, inorganic, or organic-inorganic hybrid NPs, functional packaging films with mechanical, thermal and antimicrobial properties are possible. Nanocomposites can be used to create flexible, fire-resistant, antimicrobial, and transparent barrier coatings. NPs in food packaging may also detect microbial infection. For widespread use of nanocomposites as packaging materials, further research is needed in order to extend shelf life, protect food quality, and promote commercialization.

However, due to their ultramicroscopic size, NPs are readily absorbed by cells in the human body which could have harmful consequences. Moreover, because of the increased bioavailability of NPs, toxicity is increased and could damage the immune system. As the mobility of NPs within biological systems is still unclear, silver NPs, for instance, may effectively make cells resistant to any other antibiotics. Due to their high toxicity, various other NPs, such as TiO_2 and ZnO, contribute to environmental contamination. It is thus necessary to create antibacterial NPs that are antibacterial and that do not negatively affect the environment. In conclusion, the major problem with employing nanotechnology in the food industry is that NPs are still being explored and have not yet been well described; as a result, the extent of risk that they potentially pose to biological functions is unknown and the public should be informed about the health, safety, and environmental impacts of nanotechnology as it is introduced and developed within the food system.

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Chapter 5
Biotechnology and Its Position in the Mitigation of Microbial Problems in the Food Industry

Rashmi Rawat, Mohit Sharma, and Poornima Singh

Introduction

In the Modern world, biotechnology has been devoted to every aspect of life. In foodstuffs production enterprises, biotechnology recreates a paramount part in processing food products (Dey & Nagababu, 2022). Biotechnology has various applications and aspects in the food processing industry represented in Figs. 5.1 and 5.2. The utilization of biotechnology can permit non-consumable and short-lived food items to be changed over into acceptable. Likewise, biotechnology can enhance the sensorial attributes, texture, and time span of food by stemming the maturation of microbes that provoke toxins in the food and by producing antimicrobial agents to eliminate putrefactive microbes (Miura & Okuda, 2023). The fermentation process is used to change the taste and texture of food in a desirable manner. As a consequence of fermentation, Polysaccharides are transformed into alcohols and CO_2. ELISA is also employed to determine pathogens and traces of pesticides in raw and processed food products (Sannigrahi et al., 2023). Animal-based food products are highly perishable due to their high moisture, neutral pH, and nutritional content. The processing of these foods using appropriate methods is critical to preserve their quality and microbial safety. The preservation method includes chemical, physical, and biological. In contemporary years, Biopreservation has evolved for

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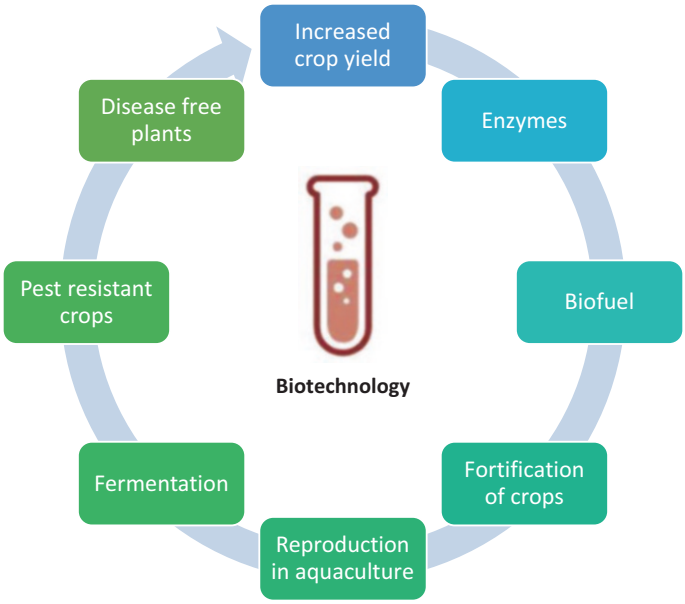
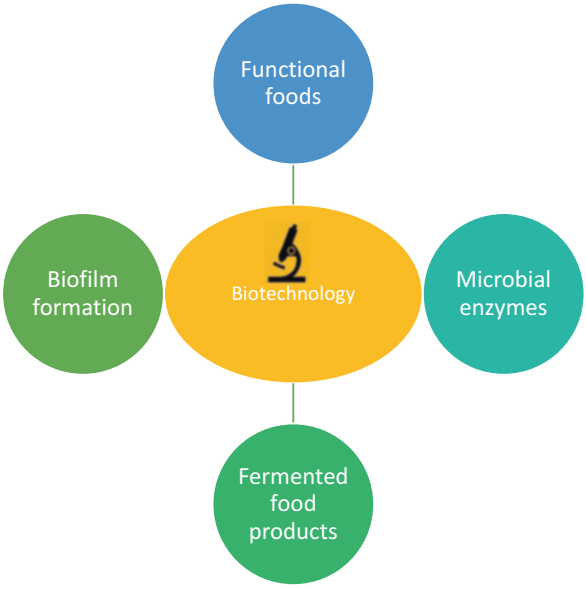


Fig. 5.1 Graphical representation for application of biotechnology in the food sector

Fig. 5.2 Graphical representation of the role of biotechnology in the reduction of the microbial issue in food industries



improvement in food security and quality attributes. Biopreservation by Lactic acid bacteria (LAB) has been used instead of chemical preservatives. Bio-preservatives are safe, enhance nutritional value, and are considered to be clean labels (Rathod et al., 2021). Biopreservation has gained special attention among alternative food preservation techniques. The utilization of biosensors for the premature discovery of mycotoxins in food developments has evolved fundamentally to stay away from monetary misfortunes and disagreeable impacts on human well-being. The three main mycotoxins producing funguses in foodstuff are Aspergillus, Fusarium, and Penicillium (El-Sayed et al., 2022). Disclosure of the above-mentioned mycotoxins might be responsible for DNA deterioration & cell cessation. An additional quantification approach has been designed for the spotting of mycotoxins in foodstuffs. Among them, biosensors have concluded up being a viable instrument for the early recognition of mycotoxins (Oliveira et al., 2019). They are profoundly delicate and easy to utilize, consequently advancing quick and reproducible breakdown. Various types of transducers and biological materials are used for mycotoxin spoilage and its early detection. Other microbial mitigation and early detection techniques in food stuff include Biofilms, Biostimulants, Bioaugmentation, Bioremediation, etc. (Nwankwegu et al., 2022). Therefore, the aim of this chapter is to highlight the role of biotechnology in microbial mitigation and its early detection in the food industry.

Microbial Contamination and Its Premature Spotting in Food Industry

Microbiological irregularity directs to the nonessential or involuntary existence of pathogenic microorganisms. Microbial contamination is driven by transmissible microorganisms (Mostafidi et al., 2020). The infectious microorganisms live & originate maturation in the processing equipment, outcomes in the preface of pathogens into the food processing phases, whereby deteriorating the food. Food contamination by microorganisms has become the greatest concern for the majority of food industries, despite the fact that food contamination also impacts food packaging. Even though reasonable hygiene procedures are sustained in industriousness, it evolves and is challenging to overwhelm contamination. By creating contaminated toxic metabolites, pathogenic contaminants can induce several disorders, including botulism, food poisoning, and other specific intestinal diseases (Sohrabi et al., 2022).

Traditional Approaches to Spot Pathogens

Expectedly, the existence of illness-causing microbes in foodstuffs can be placed through a progression of examinations, including pre-and particular improvement and specific plating, trailed by biochemical screening and lastly affirmed by

serological testing (Vidyadharani et al., 2022). Albeit the conventional technique yields precise outcomes, it requires a lot of investment to finish every one of the vital stages and arrive at a resolution. This requires a fast strategy for microbial identification that recognizes microbial investigation of food more productively and quicker than current techniques. Thus, new procedures for identifying food tainting because of uncontrolled microbial development are being created, with the conventional strategies filling in as a benchmark. The ordinary and quick identification of Salmonella in specimens of distinctive cheddar was looked at in a review. Following the previously mentioned advances, the ordinary strategy was finished, while continuous polymerase chain response (PCR) was utilized for a quick conclusion. The outcomes uncovered that the International Organization for Standardization (ISO) announced that traditional methods are slower than PCR. Since time is a significant component that should be fulfilled in the food business, a fast determination is the predominant strategy for deciding microbial presence in food varieties. In certain cases, traditional strategies have been seen to create bogus positive or adverse outcomes, adding to the method's limits (Mata & Vanetti, 2012).

Quick Analysis of Food Contamination

E-nose

An E-nose or electronic nose is a novel widget that comprises the miscellany of electronic compound detectors with halfway explicitness and an example pattern framework equipped for perceiving basic and complex smells (Dhanekar, 2020). E-nose is extremely capable of cracking down the physical and substance attributes of staples and distinguishing the bacterial impurities available in milk and milk items, which makes sense for its rising use in the food business (Yadav et al., 2023). Tin sensors in an electronic nose were used to distinguish microbial tainting in meats. Around 98% of microbes can supposedly be screened by this gadget. In some cases, electronic noses are figured out with metal edifices, which have been exhibited to stand adequate for contrasting the existence of maturation in produce. Satisfactory analysis was executed utilizing sullied cereal grain kernels to examine the nature of device outcomes & to determine the existence of Fusarium and Penicillium species in the crops. The aforementioned illustrates the significance of involving an electronic nose for a fast conclusion of contamination (Moura et al., 2023). It additionally recognizes pathogenic microbial development that is answerable for the deterioration of milk quality. Assessing parasitic spores in cereals and prepared goods is likewise utilized. Thus, the gadget can be considered for assessing food quality to recognize suspicious microbial development.

Molecular-Based Detection

To assess the microbial existence in foodstuff, genetic code successions of conceivably infectious microbes are distinguished. For the objective arrangement, reciprocal tests are hybridized. Multiplex PCR and quantitative PCR are generally acknowledged sub-atomic cycles for identifying tainting. Sub-atomic-based examinations, for instance, microarrays, loop-mediated isothermal amplification (LAMP) & nucleic acid sequence-based amplification (NASBA) can likewise identify pathogenic contamination in food (Melinte et al., 2022). The PCR cycle is adequately effective to create quicker and improved results. Progressively PCR, the method involved with deciding the pathogenic strains, requires almost 2 h after DNA extraction is finished (Gwak et al., 2020). Multiplex PCR can identify different foodborne microbes. This technique distinguishes numerous microorganisms in a single examination, making it valuable, quick, and straightforward (Chen et al., 2021). Numerous microbes were detected in shrimp. As per a review, Multiplex PCR positively detected these microbes in shrimp (Fakruddin et al., 2013). LAMP is performed isothermally with BstDNA polymerase. Bst DNA polymerase is utilized in LAMP rather than Taq DNA polymerase in PCR. Contrasting to PCR, LAMP should be possible at a consistent temperature. A few examinations uncovered that this measure has been fruitful in the location of microorganisms (Barkway et al., 2015).

Enzyme Based Immunoassays

Immunochemical assay, particularly ELISA, gauges food impurities. These use protein form arrangement. A few new immunoassay techniques have been grown as of late particularly Fluorescence polarized immunoassay (FPIA), and Sidelong stream immunoassay. FPIA considers an exact assessment of the antigen or immune response. The strategy is basic and valuable. It uses a fluorescent dye that is energized via plane-polarized light; the revolution paces of particles are not set in stone by noticing the planes. Lateral flow immunoassay utilizes immunochromatographic sticks, dipsticks, and immunofiltration. This strategy isn't just more affordable than the regular ELISA, yet it is additionally more productive and dependable. In this test, the specimen streams in a parallel heading along the solid stage. Slim power is utilized for the parallel stream. It can be handily notorious with parallel stream dipsticks, though E. coli is ordinarily identified with immunochromatographic sticks (Zhao et al., 2014). As well as identifying bacterial microorganisms, the parallel stream method can likewise recognize the existence of viruses in foodstuffs.

Biosensors and Their Role in Microbial Mitigation

Biosensors are logical gadgets that incorporate a natural detecting component with a physicochemical transducer to assemble a sign proximate to the specimen focus. As a reason for biosensor development, Ivnitski et al. (1999) investigated numerous

qualitative attributes for prompt & ambiguous confirmation of microbes, as well as miscellaneous spectroscopy and chromatography approaches (Ivnitski et al., 1999). As natural detecting components, biomolecules like compounds, immunoglobulin, genetic code, enzymes, and nucleic acids. Identified specimens incorporate explicit microorganisms, toxins, saccharides, and insect sprays. Ordinarily, biosensors are classified in the form of their primary transduction strategy. The acknowledgement signals comprise electrochemical, optical, and various transducers. Contingent upon the biochemical responses on a transducer surface and the estimating boundaries, biosensors can likewise be sorted as immediate or circuitous location frameworks. Direct-recognition biosensors are intended to identify bio-specific responses progressively by estimating the actual changes coming about because of the reagent's association. In biosensors utilizing circuitous identification, a biochemical response goes before the discovery of the response's items. Biochips and CPUs, which can recognize many particles related to foodborne and waterborne microorganisms, have gotten a lot of concentration over the course of the last years. Biochips can be intended to identify a wide range of waterborne microbes by engraving various antibodies or DNA particles against explicit microorganisms for concurrent recognition of microbes on a similar mark (Jaywant & Arif, 2019).

Surface Plasmon Resonance Based Biosensors

Biosensors established on Surface Plasmon Resonance theory are used for spotting and the predominance of microorganisms has expanded significantly lately. Miscellaneous examinations have shown that immune response immobilization by authentic adsorption is inadequate for antigen restriction. Oh et al. (2004) led a progression of investigations utilizing SPR biosensors to recognize salmonella species. Detection of Salmonella was carried out by biosensor, and the surface was wrapped with 11-mercaptoundecanoic acid SAM, followed by the maturation of a coating of protein G & immobilization of monoclonal antibodies. The gadget's responsiveness went within the limits of 10^3 and 10^9 Colony forming units/ml. Oh et al. (2004) conducted an analysis of Salmonella by employing this biosensor. The sandwich technique was utilized to recognize Salmonella serotypes. Thusly, the gadget's recognition level was between 10^3 and 10^8 CFU/ml.

Tire-Based Biosensors

TIRE is an optical strategy for concentrating on surfaces and conditions that depend upon the exploration of the amplitude and stage modifications of light. As far as total internal reflection, the chance of using stage changes of mirrored light has recently been explored. The TIRE technique was first portrayed in 1976 (Abelès, 1976). Contrasted with traditional ellipsometry and SPR innovation, deciding ellipsometry boundaries as far as complete inner reflection has altogether expanded awareness and identification levels. Consequently, the TIRE technique gives a

degree of assurance inside 5×10^7 RIU, though this record is 10^5 RIU for ellipsometric estimations (Iwata & Maeda, 2010). Since the mirrored wave is shaped on a limit between optically differentiating conditions, ellipsometric estimations give data regarding the visual design of region and the cycles impacting its optical attributes (Arwin et al., 2004; Baleviciute et al., 2013). In an analysis, the TIRE technique was employed in S. Typhimurium recognition in model specimens Starodub et al. (2011). The outcomes signified that TIRE-based biosensors exhibited higher responsiveness than the SPR. The high responsiveness of ellipsometry-based biosensors can fluctuate for various substances; however, it ordinarily arrives at a couple of nanograms. The examination doesn't need huge amounts of reagents and can be achieved with microliters of specimens. Likewise, there is a compelling reason need to name reagents, there are no harmful impacts on the object of study, the constant investigation is conceivable, the estimating range is huge (from nM to M), and the examination time is short (Qi et al., 2023).

PhL Based Biosensors

PhL is a strong innovation for the improvement of optical biosensors in light of the fact that it doesn't need bioreceptor readiness, convoluted electrical circuits, or costly gear. The working rule of PhL-based biosensors remains toward analyzing deviations in cutting-edge PhL spectra of nanoparticles brought about by the communication of organic parts. Utilizing photoluminescent nanoparticles, various substances, including particles, DNA atoms, dopamine, and S. Typhimurium, have been effectively distinguished (Liang et al., 2014; Qian et al., 2014; Viter et al., 2014). Viter et al. (2014) portrayed an innovative technique for recognizing Salmonella by utilizing biosensors with PhL of ZnO nanorods at room temperature. The outcomes showed that the communication of adsorbed proteins with the ZnO surface expanded the photoluminescent power of ZnO nanorods. A lessening in photoluminescent power was noticed following the expansion of S. Typhimurium cells and the Antigen-Antibody connection. In the scope of 10^1–10^6 cells/ml, the biosensor showed signal adjustments. As indicated by Viter et al. (2014), the biosensor's location limit was around 10^2 cells/ml. Giving surface functionalization to the covalent restricting of antibodies can possibly increment sensor responsiveness. Graphene has a grandly unambiguous surface region, electronic conductivity, thermal stability, mechanical stability, covalent holding, and polymer mixing abilities and additionally has wide applications in Biosensing and fluorescence imaging (Abdelhamid & Wu, 2013).

ISFETs-Based Biosensors

Semiconducting potentiometric gadgets such as Ion-selective field effect transistors (IsFETs) are generally utilized in biosensors. ISFET biosensors are adjusted for diverse applications, such as glucose and urea identification and clinical

investigations. The miscellaneous benefits of ISFTs are their portable size, capacity to put numerous cathodes on a solitary semiconductor chip, and modest large-scale manufacturing. Contingent upon the undertaking, various organic substances can be utilized to change the outer layer of FETs, yet most of the adjustments include catalysts, immunoglobulins, and DNA molecules. Starodub and Starodub (2000) used resistant ISFET-based biosensors effectively identify the herbicide simazine. According to the outcomes, in the wake of being treated with a corrosive arrangement and water, ISFET can be reutilized on different occasions without deterioration of signal. Taking into account every preliminary step, the examination required about 1 h.

Bio Preservatives and Their Role

Bio-preservation is characterized as the utilization of antimicrobial substances obtained from foodstuff or formed by microbial maturation towards handling and quality of food. A bio-preservative is a compound that forestalls or impedes waste brought about by chemical or natural degradation, in this way drawing out the time span of usability of an item. These mixtures are frequently obtained from normal sources, while some are shaped in food. As of late, bio-preservatives have become progressively significant because of the developing interest in nutritious and top-notch food varieties. Bio preservatives are extracted from floras, faunas, or microbes normally utilized in the aging of food varieties. Unlike manufactured or customary additives, most regular options are bio-additives; however, this term is seldom utilized. The most well-known and contemplated bio-preservatives obtained from plants, chiefly from spices, are Essential oils (EOs). Enzymes are acquired from animal items, like eggs or milk. Be that as it may, as of late there have been reports with respect to bacteriocins delivered by different microscopic organisms, including lactic acid microorganisms. Other regular additives utilized are reuterin (aldehyde) created by Lactic acid reuteri, diacetyl, and Hydrogen peroxide extracted from LAB, natural acids (lactic, malic, citrus, fumaric) extracted from various scope of microorganisms, and natamycin delivered by the few types of streptomyces. Aside from these, most food sources are safeguarded with LAB, yet ongoing investigations have shown that food microbiota can safeguard against decay microorganisms. Bio preservatives ought to be protected, modest, compelling in lower concentrations, and permissible by regulatory bodies.

Essential Oils

Essential oils, or EOs, are basically extracted by actual strategies like squeezing and refining and are perplexing combinations of aromatic and unstable mixtures delivered by plant materials like Roots, Peels, leaves, Pulp, wood, seeds, Buds, and barks. With regard to the commercialization creation of EOs, steam refining is the

Table 5.1 Major components in EOs

Plant	Major component
Oregano	Carvacrol
Clove	Eugenol
Peppermint	Menthol
Thyme	Thymol
Cinnamon	Cinnamaldehyde
Lemongrass	Citral
Coriander	Linalool
Rosemary	1,8-Cineole
Spearmint	Carvone
Fennel	Trans-Anethole

most usually utilized technique. Recent examinations in the food use of EOs it has a vast amount of antibacterial, antifungal, and antioxidant attributes. As a general rule, EOs are portrayed by their chemical structure; notwithstanding, a couple of significant constituents are liable for their antimicrobial properties. The significant parts of chosen EOs are displayed in Table 5.1**.**

EOs are chosen for their antimicrobial properties as bio preservatives; nonetheless, they ought to likewise be considered for their sensorial attributes. Thyme and oregano natural oils have shown elevated degrees of antibacterial action because of their significant phenolic parts carvacrol, thymol, and eugenol. In contrast to different substances, for example, ethers, phenolic substance's structure phenoxyl radicals that connect with alkyl substituents. Also, minor parts of EOs can have synergistic antimicrobial impacts with significant parts. Oxygen accessibility likewise impacts EOs' antibacterial activity. EOs have wide antimicrobial spectra, which can restrain or hinder Gram-positive as well as Gram-negative microorganisms. Subsequently, various food applications have been accounted for in the earlier years. In this manner, they are of distinct fascination as bio preservatives.

Mode of Action

EOs' hydrophobic properties make them especially appropriate for connecting with the lipid bilayer that makes up the membranes of microbes. As layer porousness increments, particles, and other cell parts spill out (Lambert et al., 2001), At the end prompting cell death. In addition, cytoplasmic aggravations, for example, proton thought motive force, electron flow, active transportation, coagulation of cell contents, and changed lipid-protein collaborations in the membrane (Vergis et al., 2015), are incorporated into cell rupture; the blend of these variables brings about cell death. Varieties in the compositions of natural oils should be possible hence, their wide assortment of EOs can be utilized as bio-preservatives. A few significant parts are likewise liable for cell rupture, and in this way, EOs additionally shows antimicrobial properties.

Carvacrol expands the ease of fluidity, which permits protons and potassium particles to spill out, causes membrane harm, and forestalls ATP synthesis (Ultee et al., 2002). Then again, terpenes slowed down and infiltrated the lipid structure of the cell wall of microorganisms, subsequently denaturing protein and disturbing the cell wall, which prompted cell lysis and cell death (Barry-Ryan & Bourke, 2012). The antimicrobial property of EOs, boosting the speed of the process, is not entirely settled by the cell structure contrasts between Gram-positive and Gram-negative microbes. When contrasted with Gram-negative microbes, which are more delicate to EOs since they contain lipopolysaccharide, a hydrophilic part that forestalls EO collection on the layer, Gram-positive microscopic organisms act all the more rapidly because of EOs. The grouping of EOs expected to inactivate or restrain Gram-positive microbes is normally lower than that expected for Gram-negative microorganisms. There is an absence of information on fungi species. However, the latest reports give proof with respect to the method of activity of EOs. Rosemary natural balm (0.3–1.5 μl/ml) restrained the development of Aspergillus flavus by diminishing how much ergosterol was in the cell film; ergosterol is exceptionally fundamental for mass layer capability, smoothness, and penetrability.

Applications in the Food Industry

Food compositions, for example, macronutrients, water activity, pH balance, and enzyme activity can lessen the antimicrobial impact of EOs. Low pH upgrades EO solvency and antimicrobial action. Particularly, the antimicrobial movement of EOs relies on their concentration values. However, off-flavor major areas of strength, or sometimes their odour limits their application in foodstuff. The application strategy for EOs in food is fundamental for microbial control, sensorial attributes, quality, and the time span of usability. The most appropriate application strategy relies upon the kind of food, introductory microbial burden, target microorganism, sensory effect, EO flavour similarity with food, and capacity temperature. Direct expansion (as an ingredient of food plan or on food surfaces by spraying or dipping/coating), vapor stage lemongrass, and rosemary EOs have been utilized in meat products also on some fruits and vegetables like tomato lettuce via an active packaging approach. Food-borne pathogenic microorganisms are EO targets. Every nutritional category has agent studies. For every food type, a synopsis of a few delegate studies is displayed in this section.

Fruit and Fruit Products

Natural oils or certain methods in blending with EOs have additionally been utilized to bio safeguard fruit and fruit juices endlessly. The most well-known and broadly acknowledged method for deactivating microorganisms in natural product juices includes hot temperature treatment, which can modify the juice's colour and flavour.

Hot temperature treatment at 55 °C with 190 parts for each million-orange fruit. EO in squeezed orange pulp abbreviated the time expected to inactivate the microbe E. coli O157:H7 by 2.5 times. The sensorial harm of squeezed orange was relieved by the incorporation of orange EO, which cut the hot temperature treatment time down by 50% (Espina et al., 2014). 2log reduction of Saccharomyces cerevisiae was achieved in apple-orange (1:1) juice, which was thermally treated (for 2 min at 90 °C) and shelf stored at 25 °C for 8 days. As an outcome, it helped to retain its quality attributes, colour, and flavour (Tyagi et al., 2013).

Vegetable Products

EOs can change the flavours of vegetables, which have a short time span of usability because of microbial deterioration and other variables. It is important to utilize a suitable application mode to ensure sensorial worthiness. Ponce et al. (2011) analyzed EOs to Romaine lettuce leaves in polyethylene terephthalate plate to expand their time span of usability at 6 °C. EOs incorporated samples in cases of repressed local microorganisms for 1 week. However, they were successful in maintaining sensorial attributes of microbial decay.

Dairy Products

It has been proposed that EOs could be utilized to save dairy items; in any case, mindful methodologies have been accounted for on the grounds that numerous milk items are aged or matured food sources. Thusly, EOs could hinder LAB or molds that are liable for the improvement of the flavour, smell, surface, and sensorial attributes of cheeses and yogurts. Oregano and thyme EOs at 0.1 mL/100 g (splashed on a superficial level) were viable to inactivate 5 log reduction of a Listeria monocytogenes' Likewise addition of EOs in Feta cheddar was shelf stored at 4 °C for 20 days. Following 20 days of refrigeration, both EOs helped to 4log reduction of E. coli O157 (Govaris et al., 2011). When labneh (concentrated yogurt) was shelf stored at 6 °C and 0.3% cinnamon oil was added, the time span of usability was improved by up to 3 weeks (Thabet et al., 2014).

Meat Products

Meat items deteriorate basically because of fats oxidation and bacterial development, in spite of the fact that molds might create a few issues in the development of meat products. Meats might become tainted with microorganisms during butchering or handling. LAB, a huge bacterial gathering related to meat deterioration, advances undesirable quality changes. Also, food-borne microorganisms can develop on newly slaughtered meats. Penicillium is a predominant family tracked down in developing meat products. EOs decreased and restrained microbial burden as well

as upgraded quality credits like hindered fat oxidation; EO of Salvia officinalis at low fixations (0.1%) repressed the development of Salmonella Anatum and S. Enteritidis in minced hamburger shelf stored at 5 °C for 2 weeks. Centralizations of 2% of Essential oil inactivated Salmonella up to manifolds following 2 weeks of shelf storage investigation at 5 °C for 2 weeks (Hayouni et al., 2008).

Mycotoxins Detection and Its Control

A mycotoxin is acquired from the Greek word "mykes," which implies fungus, and the Latin word "toxicum," which implies poison. Particularly, mycotoxins are low-sub-molecular weight intensifies conjoined by filamentous fungi during secondary metabolism; their chemical configuration can vary from simple 4-carbon mixtures to complex particles. The utilization of mycotoxins by humans or animals prompts obsessive or unwanted physiological reactions. At the point when mycotoxins are gulped unexpectedly, they cause mycotoxicosis (Soares Mateus et al., 2021).

30^5 fungal secondary metabolites are known to exist, among them six mycotoxins that are significant according to a farming perspective. Mycotoxin-creating parasites can grow in cereal grains under normal circumstances during handling and storage (Awuchi et al., 2022). A great many substrates and ecological circumstances are helpful for mycotoxins growth naturally (Nji et al., 2022). Particularly, the utilization of food technology essentially decreases mycotoxins yet doesn't dispose of them. Mycotoxins affect DNA, RNA, and protein union and might cause changes in physiological capabilities, including propagation, development, and advancement (Yang et al., 2020). Notwithstanding these various activities, mycotoxins might influence the digestive system, cause skin problems, make hematological impacts and diminish development.

Mycotoxin-Producing Fungi

Fungi are unicellular, polynuclear, eukaryotic organic entities that are heterotrophic and whose cells are comprised of chitin. Mycotoxins are either grown during preharvest level or throughout postharvest (storage, transport, and handling). Mycotoxins are generally created by filamentous fungi that are adapted to the earthly environment. Mycotoxins are delivered by specific types of filamentous fungi having a place with types of the genera Aspergillus, Penicillium, and Fusarium that attack crops at the field level and may develop on food varieties during capacity when they got ideal conditions for growth (Syamilah et al., 2022).

Chemical Structures, Toxicity, and Mode of Action of Mycotoxins

Aflatoxins

Aflatoxins are composed of heterocyclic mixtures that are exceptionally oxygenated. Aspergillus flavus, A. parasiticus, and A. nomius form aflatoxins B1, G1, B2, and G2. Aflatoxin B1 is the most cancer-causing type, causing liver malignant growth in humans (Baird et al., 2006). Global Office for Exploration on cancer growth characterized AFB1 as a group A cancer-causing agent (Soares Mateus et al., 2021). AFM1 and AFM2, monohydroxylated subordinates of AFB1 and AFB2, are usually found in milk (Popescu et al., 2022). AFB1 has been connected to essential liver disease in people, where it cooperates with HBV contamination to cause malignant growth, and it has been marked a group 1 cancer-causing agent in people. Insect harm might make grain crops more powerless to mycotoxin-delivering growths (Winter & Pereg, 2019). Aspergillus strains that cause aflatoxin to flourish in high dampness/humidity, hot temperatures, or potentially poor drying. This implies that grains put away in these circumstances could become contaminated (Awuchi et al., 2021).

Zearalenone

Zearalanol can cause mature adolescence in kids, and Estrogenization can be caused by the F-2 toxin (Yli-Mattila et al., 2022). Mature advancement of breasts and, furthermore estrogenic impacts in females, as well as preputial development in males, is the most notable impact of ZEA. Fundamentally rats, cows, and chickens, pigs are the most delicate and seriously impacted species.

Fumonisin

The fumonisins are a type of nonfluorescent mycotoxins which are fundamentally formed by F. verticillioides and F. proliferatum (Santos et al., 2022). Fumonisin B1 (FB1), the most predominant of the various fumonisin analogs, was designated a group 2B cancer-causing agent (Wild & Gong, 2010). The cancer-causing effects of fumonisins have all the irrelevant of being inconsequential to DNA interaction (Coulombe, 1993). Its closeness to sphingosine proposes a potential job in the biosynthesis of sphingolipids (Shier, 1992). Sphingolipids are subsequently restraining sphingolipid biosynthesis causes serious cell activity issues. In specific human populations, fumonisins are associated with causing esophageal growth. No matter what their impacts on humans, fumonisins particularly cause liver harmfulness by slowing down sphingolipid metabolism, their essential method of activity.

Patulin

P. expansum, typically comprehended as the blue mould pathogen of apples and some climacteric fruits, is nature's direct patulin producer. Patulin is excessively harmful to plant and animal cells and should react with the terminal sulfhydryl groupings of proteins in edibles (da Silva et al., 2022). Patulin deters DNA synthesis and hampers immunosuppression. Patulin exposure is coupled to immunity, digestive, and neurological disorders. (Esheli et al., 2022).

Methods for the Detection of Mycotoxins in Foods

Chromatographic Techniques

- Thin-layer chromatography

The standard TLC approach is viewed as an adequate evaluating device for the existence of aflatoxins and a solid analytical measurement procedure. TLC is a broadly involved strategy for quantitative estimations of mycotoxins utilizing fluoro-densitometry and visual systems with a detection limit of up to **0.01** ppm. Silica gel is the base of the TLC approach. Silica gel is usually infused with organic acid and has allegedly been employed to determine normal mycotoxins like aflatoxins, citrinin, and fumonisin (Pradhan & Ananthanarayan, 2020).

- HPLC

HPLC is the considerably well-known chromatographic process for quantitative investigation of mycotoxins, particularly aflatoxins. Various kinds of solid stage or reverse stage columns, elution combinations and gradients, identification techniques, and test preparation and purification methodologies determined these HPLC strategies. Unique mixtures are isolated on the basis of their association with the column matrix and the mobile phase solvent by infiltrating dragged samples into a standard or reverse-phase HPLC chromatography (column (Vaudreuil et al., 2020). A few mycotoxins, such as ochratoxin and citrinin, have biological fluorescence and can, in this manner, be identified straight by HPLC-fluorescence identification (Singh & Mehta, 2020).

- LCMS

Liquid chromatography combined with mass spectrometry LC-MS) or couple mass spectrometry LC-MS/MS) is an adequate technique for mycotoxin recognition and recognizable proof, particularly for those toxins with low UV/Vis absorbance or local fluorescence (Tsagkaris et al., 2019). Flores and González-Peas (2017) detailed the concurrent measurement of **16** mycotoxins in cattle milk, including aflatoxins M**1**, B**1**, B**2**, G**1**, and G**2** (Leite et al., 2021).

- Gas chromatography-mass spectrometry (GCMS) technique

Gas chromatography can be utilized to see whether mycotoxins are sufficiently volatile at the column temperature or can be modified into volatile subordinates. It is, in many cases, utilized in additional specialized labs to analyze certain mycotoxins, particularly type-A mycotoxins, which are not handily examined with HPLC (Luo et al., 2022). It tends to be combined with various discovery frameworks; however, most continually electron-capture detectors (ECD) and mass spectrometry (MS) are utilized (Lobato et al., 2021).

Immunological Methods

- ELISA

ELISA operates because an essential antibody that is well-defined for the toxin of interest or a marked toxin-enzyme conjugate form contends with the toxin in the specimen for a set number of restricted sites. The subsequent complex reacts with a chromogen, which can be estimated with a reading (Chu, 2019). ELISAs are ordinarily profoundly unambiguous, fast, and generally easy to use. Delicate microtiter plate immunoassays (in ELISA design) are financially accessible. Most of these units depend on a heterogeneous ELISA design in which the specimen toxin rivals a marked toxin (for example, a toxin enzyme conjugate) for a predetermined number of antibody-binding sites. More toxin is available in the specimen, the more vulnerable the limiting of the marked toxin and the more vulnerable the sign created by the assay (Geleta, 2022).

- Fluorescence polarization immunoassay

This strategy is predicated on the contest between free and fluorescein tracer-toxins for toxin-explicit monoclonal antibodies in the arrangement. In this procedure, no enzymatic response is expected for discovery. Besides, recognizing the bound and free labels is pointless. Such a measure has been produced for evaluating aflatoxin in the grains (Zhang et al., 2022).

Biofilms in Food Industry: Mitigation of a Microbial Issue by Introducing Biofilms in the Food Industry

Biofilms

These are assorted biological systems created by single or multiple organisms submerged in a superficial environment of moving based on the type of nutrient-supplying environments and the varieties populating it. Fungi and Bacteria are instances of microorganisms that can develop these biofilms. The existence of numerous forms of bacteria holds critical conservation benefits since this stimulates

biofilm association with a surface. This could occur without any specific fimbriae in certain species. Antimicrobials like quaternary ammonium complexes and other biocides are more impervious to blended biofilms (Meyer, 2015). The extracellular network comprises polysaccharides, polypeptides, and nucleic acids. The aforementioned framework might be converged to tough surfaces (Tools, carriers, conveyance, biological stages and soil etc.) (Flemming et al., 2016). The superficial environment grid plays an underlying part, which represents the biofilms' wonderful steadiness in food enterprises. It makes convoluted inclinations for oxygen and supplement transport, contains extracellular enzymes for feed and food, permits cell correspondence particles to be moved, and safeguards the implanted cells from hurtful synthetics. Biofilm-related impacts (host specificity, rusting of metallic surface, change in organoleptic qualities because of lipase or protease digestion) are essential in particular enterprises, for example, dairy creation lines, where various designs and processes (Milk tanks, pipes, cheese capacity tank, pasteurizers, and packaging methods) capability as outer reactants arrangement at distinguishing temperatures. It might be comprised of S. enterica, L. monocytogenes and pseudomonas species (Mizan et al., 2015).

Its existence in processing industries can emanate toxins. It can degrade compositions, generating personal or innumerable intoxications. This is why Biofilm networks in a food-production area jeopardize human well-being.

Bacillus Cereus

Bacillus cereus is a facultative anaerobic or spore-framing Gram-positive bacteria that can flourish in different environments and temperatures (4–50 °C) and be thermal safe. Compound treatments, and sunlight (Bottone, 2010). The staying power of vegetative strains of B. cereus on food equipment surfaces is risky to one's well-being. Moreover, because of the formation of endospores, this bacterium is equipped for enduring modern pasteurized activities. This hampers biofilm expulsion utilizing cleaning procedures (Auger et al., 2009) and can charm biofilm perseverance in milk plants, affecting dairy and its product storage of realistic usability (Gopal et al., 2015). Biofilms of B. cereus are habitually found close to various microorganisms in the industry (Majed et al., 2016). Their linkage is served by their perplexing association of polysaccharides, polypeptides, and nucleic acids (Vilain et al., 2009). The paramount relationship of these strains has a prerequisite influence this permits other bacterial species to connect rapidly, which would be cleared by various streams (Marchand et al., 2012). These strains are vastly discovered in dairy processing plants (Ehling-Schulz et al., 2015; Ruan et al., 2015).

An analysis of the processing plant creating sterilized milk in North America discovered around 6% of these had a 10^6 -colony forming unit of B. cereus per ml of tested specimens & that around 5% of these entities brought enterotoxins at decks that could lead to foodborne illness. B. cereus could deliver enterotoxin in this pasteurized milk after 1 week of storage. Essentially elevated B. cereus bacterium was found in items with a high butterfat content or those that were sterilized at increased

temperatures for a brief period (Saleh-Lakha et al., 2017). Customary markers, like oxygen-consuming settlement development and psychotropic counts. In an analysis utilizing sterilized milk, 18 B. cereus isolated were recognized.

E. coli

These strains are usually found in the gastrointestinal tract and don't represent a well-being risk. In any case, a few sorts are hazardous foodborne microbes that can be transported through the water, milk & milk products, and meat & meat products. These items might have been tainted at the starting place or during the course of food processing. This contamination might happen in the food products during the pre-gather stage because of the utilization of spoiled water while creating the yields. This disease may likewise happen in storage and handling conditions, where it might emerge during the cleaning and handling of raw materials, yet additionally attributable to storage temperatures that permit the flow of bacterial contaminants to replicate rapidly (Carter et al., 2016). A few examinations have shown that kinds of E. coli may stick to many surfaces, together with steel material, Teflon, and polystyrene. (Van Houdt & Michiels, 2010).

Listeria Monocytogenes

L. monocytogenes, a Gram-positive bacteria, is typical and causes serious foodborne illness. It isn't, notwithstanding, impervious to pasteurization techniques (Milillo et al., 2012). Fish, milk items, frozen yogurt, organic products, delicate cheeses, sugar-coated candies, and raw milk are a few instances of packed food sources known to convey this illness (Rothrock et al., 2017).

Biofilms of these strains are generally made of teichoic acid and shall foster on various surfaces across a food processing plant. These contaminations can further reproduce at lower temperatures (Silva et al., 2008). There is a record of five outbreaks that occurred due to Listeria monocytogenes from 2014 to 2016 (Burall et al., 2017). The presence of these strains demonstrates remarkable fidelity to numerous surfaces and restraint to chemical compounds, which are significant justification behind microbial migration reactions in the milk and meat products.

Advanced Biotechnological Tools for the Control of Microbial Mitigation

Biotechnology is the use of technology and science to living creatures and their components, products, and models to change living or non-living elements and produce knowledge, products, and services. The diversity of plants and microbes makes

it more difficult to improve applied genetics (Alan, 2023). The successful gene modification of microorganisms has encouraged agricultural scientists to employ plant breeding technologies. The strategy of the plant breeder is determined by the specific biological elements of the crop being bred to facilitate the emergence of fresh varieties and sometimes even types of plants by evading current biological barriers to the interchange of genetic material. Emulsification offers food products a distinct look and sensory qualities that substantially influence customer perception. Because human perception is complex, food manufacturers typically require numerical criteria for quality control (Andreani et al., 2023).

Bioactive chemicals derived from natural sources are frequently incompatible with the food matrix, break down quickly during food preparation, and are vulnerable to digestive action in biological systems. As a result, these bioactive compounds are encapsulated for improved protection and simpler integration into the food matrix. Because of the acidic environment in the gastrointestinal tract, bioactive proteins disintegrate quickly and are digested by hydrolytic enzymes. Furthermore, hydrophobic chemicals, such as polyphenols, have a low water solubility, which makes integration into food and absorption in the digestive tract difficult (Maurya et al., 2023).

Bioremediation

Bioremediation is comprised of two words: “bios” (living animals) and “remediate” (to cure an issue). Bioremediation is a successful innovation/process that bridles the capacity of organic living beings to break down environmental contamination, subsequently helping the environment in supporting regular environmental factors. Bioremediation strategies are constituted of two approaches in-situ & ex-situ (Kirthi & Chaudhuri, 2023). The strategy for treating defilement in its local area under natural circumstances is known as in situ. However, the ex-situ method includes eliminating the pollutant and treating it far away from its local site, for instance, under research facility conditions. Bioremediation is an earth-useful, low-support, minimal expense, low-input, and long-haul way to deal with polluted site cleanup (Kumar et al., 2022).

The utilization of living creatures for eliminating pollutants depends on the possibility that organic entities feed on impurities for their development and digestion and thus can eliminate compounds from the environment. Many microbes, such as bacteria and fungi, degrade complex compounds relatively efficiently, and the resulting chemicals are usually safe for food. Fungi, on the other hand, can digest vast complex organic substances that are generally not digested by other creatures using their hyphae. Similarly, other biological creatures such as protozoa, algae, and plants have been discovered to be capable of absorbing nitrogen, phosphorus, sulfur, and a variety of minerals from contaminated foods (Raj & Das, 2023; Kirthi & Chaudhuri, 2023).

Bioaugmentation

Bioaugmentation is a novel approach to utilizing the ability of various microorganisms in their natural environments to clean up contaminants from polluted regions. The method employs the isolation of natural microorganisms (fungi, bacteria, etc.) and their eventual addition to contaminated locations after cultivating them under ex-situ conditions to increase their abundance and functional metabolism to remediate pollutants such as metal (Rafeeq et al., 2023). Bioaugmentation can be laid in the accompanying ways: (a) by dragging local pathogens from the objective tainted site and refining them under lab conditions before re-immunization; (b) dragged pathogens are not intrinsically inoculated to the wellspring of the local culture, and (c) extraction can be incorporated to the site with the assistance of crafted and transformed microorganisms (Raj & Das, 2023).

Biostimulants

To boost the metabolic step of a competent microbe for efficacious pollutant debasement, favorable changes in some parameters, such as oxygen, water, pH change, accessible phosphorous, and nitrogen supplies, must be utilized to boost the bioremediation process. (Triozzi et al., 2023). Biostimulation, which involves the addition of nutrients, has the promise of speeding up deterioration rates. Biostimulation is the arrangement of supplements, oxygen, pH and temperature changes, and different variables to the defiled site to build the mass action of local microorganisms reasonable for bioremediation. Thus, Biostimulation speeds up the pace of decay in dirtied areas by bringing restricting supplements into the arrangement of the microbial local area that lives there. Biostimulation assumes a significant part in making good circumstances, especially in enacting microbial populaces that have adjusted in their territories because of steady toxin openness at contaminated areas (Stincone et al., 2023).

Conclusion

The rise in popularity of genetically modified crops that are resistant to herbicides, insects, and pathogens has resulted in increased crop yields and decreased production costs. The primary focus of modern biotechnology is on the development of foods that exhibit better nutritional, taste quality, and safety characteristics. Biotechnology, as a holistic field, offers a wide of innovative devices which enable the detection of microbes and their toxins. These devices comprise several biosensors, Protein Misfolding Cyclic Amplification (PMCA), and ELISA analysis. The

PCR technique has been constructed for the purpose of identifying the presence of contagious pathogenic agents, including bacteria, viruses, fungi, and other relevant micro-organisms. Various researchers have claimed that the utilization of biotechnology in modern food production can results in the provision of in the wider range of nutritionally enhanced, healthier, more palatable, secure, long-lasting, safe, and convenient food products to global populations, all at lower costs.

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Chapter 6
Food Laws and Regulations Related to Food Security

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Introduction

Food is the basis for sustaining a healthy life. A nation's growth resides in its healthy living population that contributes flawlessly to achieve overall growth. The journey of food from "Production to consumption," "Farm to fork," "Unstable to the table," and "Boat to the throat" are the activities where these agencies abide by their laws and regulations. Globally, the average food supply is 2881 kcal/person/day against the average dietary energy requirement of 2353 kcal/person/day (FAO, 2014). Every country requires laws and regulations to produce wholesome food commodities and prohibits the sale of unsafe food products that would jeopardize living beings. With the increase in population, the demand for agricultural Production has also increased. However, the increment in the Production of agricultural products is associated with the broader utilization of chemicals. During food transport, its protection and storage also depend on the usage of chemicals. The chances of contamination are increased with the processing of foods in large quantities.

Import and export of food commodities need regulations at national as well as international levels. These laws and regulations, along with food control organizations, ensure the safety of food commodities that are imported, exported, and produced at a domestic level. Food safety regulations are increasingly witnessed skeptically from an economic point of view and provided with the performance and information criteria, generating pressure for effective laws and regulations. Food safety regulations, however, unabatedly focus on process-based requirements and ensure that product liability systems efficiently support food producers, processors,

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and distributors to deliver products comprising safe acceptance. A nation experiences food shortage due to various unfathomable reasons at micro and macro levels, such as environmental degradation, disruptions in food supply chains, and unfavorable food production due to severe weather conditions, economic crises, and various diseases (FSSAI, 2020). Climate change, such as heat waves, cold waves, droughts, and floods, also aggravates food security and Production (Szabo et al., 2016, 2018). Lesk et al. (2016) estimated that droughts and heat waves could lead to an approximately 10% decrease in crop production nationally (Lesk et al., 2016).

The social and economic impacts of the 2020 Covid-19 pandemic hit developing countries more severely. In India, the pandemic situation led to disruptions in the food value and agriculture chain, leaving the population with food scarcity. The WHO guided people with preventive measures wearing face masks in public, frequently washing hands, periodically using sanitizer and social distancing. Lately, people also gotten vaccinated against this pandemic variant. The FDA and WHO issued guidelines for food business operators to combat the novel coronavirus. In India, FSSAI issued the same guidelines for food business operators to reduce the risk of their proliferation (Gopalan & Misra, 2020). The FSSAI and international organizations implemented the guidelines about healthy and nutrient-rich foods for home quarantines and for people who were at risk of getting COVID-19. FSSAI released preventive measures for food hygiene and business regulations (Gopalan & Misra, 2020). However, The COVID-19 pandemic has been observed to result in global poverty unevenly and lead to an economic slowdown. The pandemic negatively affects global food supply and security (SDG-2) and might be persistent. (Parmeshmwar et al., 2020).

Pesticide poisoning has become a significant concern for its deteriorating effect on living populations worldwide. In developing countries, disproportionate deaths are caused by pesticides and their residues due to their misuse, poorer regulation, a deficit in surveillance systems, inadequate, inaccessible information systems, etc. Protective measures are limited for pesticide users and applicators (FAO, 2003). The United Nations Food and Agricultural Organization (FAO) developed the International Code of Conduct (1985) on pesticide distribution and application to create harmonic agreements between pesticide-exporting and importing countries.

Structure of Food Law

The primary food law ensures consumers pure and wholesome foods and that the food is safe and produced under sanitary conditions (Radomir, 2009). Food law includes all rules and regulations, and rather than doctrinal distinctions, it focuses on societal applications. It consolidates public, private, national, international, criminal, and administrative provisions (Vander Meulen, 2014). An essential part of this law is defining it in various terms, including food, natural food, imitation food, food additives, adulteration, pesticide residues, food contaminants, and so on (Radomir, 2009).

In most countries, the Adulteration of Food and Drink Act of 1860 and the Food and Drug Act of 1872 have had a significant impact, which manifests the central position of 'adulteration' in the present era (Vander Meulen, 2014). The United Nations (UN) focuses on human rights. The FAO and WHO include risk assessment and management. The World Trade Organization (WTO) significantly applies food standards in trade and resolving disputes. The WHO operates risk communication structures and is highly influential in incident management. International food law is a meta-framework (Vander Meulen, 2011). Food Law can be divided into two parts: a fundamental food law, and regulations.

The fundamental food law carries broad principles, while regulations include detailed provisions that govern the products of different categories and come under each set of regulatory authorities. The primary food control law includes food standards with hygienic provisions, food additives, tolerance against chemicals, etc. Detailed provisions are necessary to effectively administer enlightened compliance with the fundamental food law. The legislative branch of the government passes the fundamental law, and the executive or administering agency elaborates the detailed regulations and puts the law into effect. Including the specifications of food processing and standards, hygienic practices, food additives and pesticides, packaging, labeling, and prompt regulatory revisions become necessary because of new scientific knowledge. Such modifications can be made much more expedited by executive agencies rather than using legislative bodies (Radomir, 2009).

Food standards are part of the regulations in some countries, while in other countries, they are legislated separately. Regardless of being a part of regulation or separate legislation, food standards become part of the enforcement structure, thereby implementing a food law. The following points should be taken into consideration while including the principles in the fundamental food law:

- Scope of the law and its primary purpose.
- Definition of basic concepts.
- Competence in law implementation.
- Inspection and analytical procedures.
- Enforcement and penalty procedures.
- Rules and regulations for additives, pesticides, and contaminants.
- Packaging and labeling.
- Procedures for preparing and amending the regulations for implementing the law (Reddy et al., 2017).

Food law prohibits importing and distributing adulterated food commodities and falsely labeled food products. The adequate implementation of such a law promotes fair trade practices through compliance with its basic provisions, which can keep the manufacturer and dealer from unfair competition and lead to the development of the food industry since quality control tends to promote fathomable acceptance of foods at the commercial level. As per the US Food, Drug, and Cosmetic Act, a food exhibits adulteration if:

- It is contaminated with toxic substances that make it injurious to health.
- A raw agricultural commodity contains pesticide residues that are not legalized by the US Environmental Protection Agency (EPA) or in excess of tolerances established by the US EPA regulations.
- Any part of the food is decomposed (Radomir, 2009).

The fundamental food law should describe the exact content of offenses that can lead to penal action. Such offenses may be:

- Deliberately adulterate the food product.
- Marketing of foods containing unlawful or unauthorized constituents.
- Fraudulent use of labels and trademarks.
- Poorly satisfying standards laid down by the law.
- Hygienic requirement violation (Radomir, 2009).

The FAO and WHO have introduced a Model Food Law (FAO, 2018). The contents of the Model Food Law can act as a template for developing the national law.

FAO/WHO Model Food Law Contents

I. Preliminary
   (a) Short title and commencement
   (b) Interpretation

II. General provisions
   (a) Prohibition of poisonous or adulterated food
   (b) Deception
   (c) Food standards
   (d) Production of food under insanitary conditions

III. Importation and warranty
   (a) Import
   (b) Warranty
   (c) Defences

IV. Regulations regarding food safety and standards
   (a) Regulations

V. Administration and enforcement
   (a) Food Standards Board
   (b) Role of authorized officers and their duties for official laboratories
   (c) Ministry authority in obtaining particulars of specific food ingredients

VI. Legal procedure

(a) Role of the judiciary to cancel the illegal license and to dispose of the articles
(b) Prosecution
(c) Penalties
(d) Certificates of analysis (FAO, 2018)

Food Safety Regulations

Food safety regulations generally include the following measures:

- General rules and regulations
- Food safety standards
- Hygiene
- Food additives
- Pesticides and their residues
- Food packaging and labeling
- Food advertisement (Radomir, 2009)

Food safety controls and regulations are most prominent in developed countries but can also be observed in developing countries. Food safety regulations are attributed to many aspects, including the measures employed in the establishment of regulations, the private and public food safety control system relations, the government approach, the private parties' response to a regulation, and the food safety regulation implications in trade (Jairath & Purohit, 2013). Regulatory decisions should be constant across different food safety aspects, for example, food safety protection from the environment in transportation. The risk assessment principle has been preserved in the procedures for operating international standard organizations, for example, Codex Alimentarius and the SPS (sanitary and phytosanitary) Agreement of the World Trade Organization (WTO).

There are fast-developing food safety aspects, for example, genetically modified organisms, for which the scientific understanding level needs to be revised to embark on thorough risk analysis. There is no declaration that can manifest the performance of the government in such circumstances. However, it has been suggested that precautionary measures should be adopted to determine an accurate protection level. The economic justification or rationalization for food security measures is based on the social optimum risk level, at which the marginal costs and benefits of changing different food safety levels are equated. It has become operational by analyzing regulatory impact, which is systematically a measured assessment of the costs and benefits of suggested regulations. It was popularised by the OECD (Organization for Economic Cooperation and Development), among which most members applied regulatory impact analysis in some form.

The Public Sector's Role in Developing and Developed Countries

Standards of Food Safety

Food safety standards positively contribute to making consumers concerned over food safety, social coverage of food safety failure, globalization of food value chains, and food testing technology and epidemiology innovations (ITC, 2020). To satisfy the exportation requirements, food standards followed by developing countries are similar to those applied in developed countries (Hoffmann et al., 2019).

Food Safety Levels and International Trade

Several global public capacity levels implicate that substandard food may be exported illegally to countries with a deficit of vincible border inspection systems in enforcing food safety regulations. A research study has evaluated the effective Prohibition of Chinese milk products enacted in Tanzania during melamine poisoning in China. They analyzed that the Prohibition did not keep contaminated milk powder from being commercially sold through various channels (Manning & Soon, 2016; Schoder, 2010). Trade increasingly affects the safety of food commodities available to customers when the imported food is better than the native food in terms of its sanitary quality. It has been evaluated that the percentage of aflatoxin present in corn imported from the USA and Argentina is nearly seven times lower than that in corn produced in Indonesia (Minot et al., 2015; Tangendjaja et al., 2016).

Strict food regulation and controls in export markets enhance the food security and safety roles of enterprises that serve domestic market needs. This implies that exporters' compliance with strict food security measures may positively affect the food supply at the domestic level (Hoffmann et al., 2019). However, it has been shown that the HACCP systems adopted by fish exporters in Brazil did not show any enhancement in food safety at the domestic level (Donovan et al., 2001).

Food Safety as per International Perspective

The International Standard ISO 22003 defines a food safety and management system (FSMS) as a set of interrelated elements in establishing and achieving the objectives of directing and controlling food security and safety organizations. The critical elements FSMS includes are:

- Good practices
- HACCP (Hazard Analysis and Critical Control Point)
- Management system
- Regulatory requirements and communication

Globally, most food industries employ HACCP to achieve food safety. However, it is impossible to encounter all the issues regarding food safety, such as issues caused by agrochemicals, pollutants, natural toxins, etc., through HACCP alone. Besides HACCP, FSMS has become more popular at national and international levels since it systematically adopts new scientific measures and appropriate food safety regulations enforced by the National Food Authority (Attrey, 2017).

Government's Role in Regulating and Enforcing Food Safety

The supply of safe food is associated with both reproductive knowledge and the enforcement of equitable law. New laws and regulations must be made effective intermittently to protect unabated provisions of food products that are wholesome and safe for the living population. The all-embracing goal of the Food and Drug Administration (FDA) in most countries is to be responsible for compliance with food safety laws ensuring three main objectives of protecting public health.

1. Citizens must be updated with the nutritive components of essential food products.
2. Ensuring a safe food supply by enforcing the existing laws on the food industry.
3. Investigate the toxic compounds in order to eliminate them and monitor the food supply chain regularly to prosecute economic fraud.

It is necessary to bring out the enforcement of the laws once they are enacted to ensure compliance by the entire food industry, which also includes such industries connected with the food source, packaging, labeling, transportation, distribution, and retail. Resources and authority are given to FDA to write down the regulations and assemble employees and consultants, to inform, enforce, and eliminate any risk related to food safety. The governmental authorities associated with the supply of potential food tend to be given resources and authorities to discharge the duty of informing, enforcing, and eliminating as described above.

The collaboration of other government agencies is also required in addition to FDA. The US Environmental Protection Agency (EPA) promotes pure drinking water, nonpolluted air, and nontoxic natural resources; the US Department of Agriculture (USDA) ensures plant and animal well-being and wholesome food services; the Immigration and Customs Enforcement of the US Department of Justice are also included in banning the contaminated and illegal substances. Therefore, sharing the information and database between authorized agencies is a means of necessity for food safety enforcement (Johnson, 2015).

Reproductive knowledge is the primary basis for setting the protocols to enforce food safety regulations equitably and informing, enforcing, and eliminating unwholesome food commodities. Risk assessment is a scientific process that concerns food contamination from a fair perspective. The scientific risk calculation favorably estimates the actual risk involving available and current information.

Tools and Programs Ensuring the Security of Food Supply

Generally, 95% assurance of detection of microbial or chemical agents present in food is provided by periodic food monitoring if it occurs in more than 1% of product lots. The food monitoring system investigates and controls the movement of potentially contaminated products. The executive branch of the government authorizes the field inspectors with the power of the agency. Some food products are anonymously monitored and tested if they have false labeling. Contaminants must be retested in individual samples and handed over to more than one laboratory separately to assure fairness if used beyond their permissible limit.

The food safety officer inspects food products from the package to its distribution and storage. The discretionary report of the "accidental" exposure program has done a fair job in various countries. The various products of food possibly get contaminated with pesticides, natural, microbial, industrial, or chemical toxicants. In such cases, the manufacturer probably reports to FDA which in turn refers a trained consultant to provide assistance to the food industry. Consultant fees are paid by the company owner that depends on the nature and extent of the consultation.

The Food Safety and Inspection Service (FSIS) in the US ensures the implementation of the current and future safety of the food supply. Regular, surveillance, monitoring, and voluntary reporting are all included in risk management that tends to declutter the problems and promise a safe food supply (FDA, 2017). The US FDA has initiated the Hazard Analysis & Critical Control Points System to target the risks related to food safety more adequately, and the inspecting resources' allocation would be enhanced further (FDA, 2017).

Food Security in India from the FSSAI Perspective

India encounters various challenges in its quest for food safety (Umali-Deininger & Sur, 2007). Contaminants at the farm level, such as pesticides and toxic waste, and contaminants at manufacture-level, such as using additives in excess amounts, chemicals, adulterants, unhygienic processing of food, etc., make it unsafe for consumption (Fung et al., 2018). Therefore, every step sets a challenge to food safety regulation enforcement. In India, food safety was encompassed under 8 acts and orders by the authority of various food ministries and departments (Reddy et al., 2017). However, all the standards, regulations, and enforcement procedures required a single reference point (Sushila, 2020). In 2006, the Food Safety and Standards Act (FSSA) was enacted and it replaced eight laws that were operational before (Johnson, 2015).

1. The Prevention of Food Adulteration Act, 1954 (37 of 1954)
2. The Fruit Products Order, 1955
3. The Meat Food Products Order, 1973
4. The Vegetable Oil Products (Control) Order, 1947
5. The Edible Oils Packaging (Regulation) Order, 1998

6. The Solvent Extracted Oil, de-oiled Meal, and Edible Flour (Control) Order, 1967.
7. The Milk and Milk Products Order, 1992.
8. Any other order issued under the Essential Commodities Act, 1955 (10 of 1955) relating to food.

FSSAI governs compliance with food regulations in India, enacted by the FSS Act, 2006, and operationalized with Food Safety and Standards Rules, 2011 notification, along with six regulations that were enacted on 5th August 2011 (Dhara et al., 2021). It governs the regulation from food manufacturing to import with prescribed food standards. It prohibits misleading advertisements, and illegal trade practices for promoting sales. It brings awareness among consumers and ensures wholesome food to consumers (Goswami & Mulherker, 2012).

FSSAI Standards

FSSAI frames the regulations and signifies standards for all food products inspected by 21 Scientific Panels and one Scientific Committee consisting of various independent experts and scientists (FSSAI, 2021). Food standards are re-evaluated to consider the food science and nutrition developments occurred lately and also considered different consumption patterns, new additives and products, advanced processing and technology, analytical methods, the manifestation of new risks, and feedback from all stakeholders. FSSAI notified 21 regulations for effectively implementing the FSS Act, 2006, through which different food standards and control of food business by issuing licenses, prohibiting and restricting certain product selling, fixing maximum contamination levels, sampling procedures, imported products, approving non-specific food and ingredients, food safety auditing, recognition and notification of laboratories, packaging, and distribution of balanced diets for children in school, infant food, labeling, packaged product display, etc. are mentioned (Dhara et al., 2021).

The Bureau of Indian Standards (BIS) follows ISO 22000: 2005, which allows all types of food chain organizations to implement FSMS, which is a little more comprehensible than the HACCP. FSSAI and the State Food Authorities maintain a system of controls involving risk communication, food safety surveillance, and other monitoring activities that cover all the food business stages. The Act also encourages the Food Authority to conduct food safety audits based on FSMS (Attrey, 2017).

Registration and Licensing

The food business operators must register under FSSAI as per their Production or capacity to start any food business. By registering under FSSAI, food business operators must conform to all FSSAI regulations. FSSAI has unabatedly been

facilitating ease of carrying out business, such as implementing cloud-based technology and an open-source Food Safety Compliance System environment to issue licenses (Dhara et al., 2021).

Food Testing

Food testing needs a group of laboratories carrying basic modern analytical facilities and technical manpower. Hence, FSSAI consolidates systematic education and capacity building among employees, regulators, and the general public (FSSA, 2006). It notifies NABL accredited laboratories to carry out food product analysis. There are 190 notified and 19 referral food testing laboratories, and 12 laboratories has been recognised as National Reference Laboratory (NRL) and 2 laboratories as Ancillary National Reference Laboratory (ANRL) out of these 209 laboratories in setting up the procedures and validating testing methods, developing new methods and ensuring proficient testing in the food laboratories. It has launched an online portal named as Indian Food Laboratories Network, where various stakeholders can manage all food testing activities online. It has also empowered its staff by recruiting several posts, including Central Food Safety Officers (CFO). Furthermore, Central Licensing Authority (CLA), which was associated with State Authority, can take legal action if any food business operator (FBO) does not conform with the FSS Act, 2006 and its regulations (Dhara et al., 2021).

Eat Right India Movement

The Eat Right India movement assures the national population of safe and wholesome food. It comprises various programs. The main motive of the initiatives of Eat Right India movement is to demand and supply wholesome food commodities in a safe manner. The initiative related to supply develop food business capacity buildings to promote self-compliance, while the demand-based initiatives motivate consumers to demand safe and sutainable food. FSSAI has initiated a Food Safety Training and Certification (FoSTaC) program that ensures the availability of a trained and certified Food Safety Supervisor (FSS) in every food business premise. Eat Right Station, BHOG (Blissful Hygienic Offering to God), Clean Street Food Hub, and Clean and Fresh Fruit and Vegetable Markets are picked out to many sellers. The scheme of Hygiene Rating has been initiated for establishing food services such as restaurants and cafeterias, bakeries and confectionaries, and meat shops. FSSAI works on developing awareness among consumers, testing adulteration, and ensuring healthy choices. The efforts to sustainably produce and consume food commodities are made to promote eco-friendly food practices and rituals (Dhara et al., 2021).

Food Securities

The exponential rise in population has led to increased food demand, which was met by combined scientific and technological advances, institutional intervention, government policy, business investment, etc. (Cole et al., 2018). FSSAI plays a significant role in controlling the Import of food products. Moreover, FSSAI has initiated an Indian Food Sharing Alliance (IFSA) to resolve wastage of food and hunger crisis issues by integrating, food recovery organizations, agencies, and NGOs. Programs such as integrated child development services (ICDS), mid-day meals, food-for-work (FFW), public distribution system (PDS), antyodaya anna yojana (AAY), etc. have also been encouraged to increase the nutritional values of food products with micronutrients to cut down malnutrition among the population (Dhara et al., 2021).

Challenges of FSSAI

FSSAI is still struggling with adequately implementing the FSS Act, of 2006, even though carrying a legal framework. The regulatory staff is insufficient at the state and central levels. Food Safety Officers, the pillars of the FSSAI, are fewer in number than desired. Furthermore, more laboratories are needed in the country. The number of laboratories per million people in India is much lower than in the United Kingdom, Germany, the United States, etc. There is also an urgency of upgrading the food testing laboratories. There are a multitude number of unorganized sectors that requires accessible internet facilities, which makes them unaware of the rules and regulations made under FSSAI (Dhara et al., 2021).

Reddy et al. (2020) evaluated the food safety standards followed by street-food sellers in Hyderabad and Delhi in 2017 after getting introduced to the FSS Act, of 2006. They found that only about 1/3 of the street-food sellers had registration under this act to carry out their shops, and most food sellers were not conforming to basic food safety principles that include using aprons, water, and soap for cleaning utensils, and many were deficit in refrigeration facilities (Reddy et al., 2020).

FSSAI was set up to consolidate all food products. However, some products need a BIS license, such as dairy food for infants, milk cereal-based and processed cereal-based weaning food, packaged drinking and mineral water, etc., and AGMARK certification such as multi-source edible vegetable oil, fat spread, etc. besides FSSAI. Food business operators, including manufacturers, processors, retailers, wholesalers, distributors, consumers, and even officials, lack food safety awareness and also need to be better at understanding the rules and regulations that are updated constantly (Wertheim-Heck et al., 2015). The biggest challenges that are faced by food industries are poor information and clarity of regulations (Dhara et al., 2021).

Implications of COVID-19 on Global Food Security

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019 resulted in the widespread of a viral strain named COVID-19, causing severe respiratory illness. COVID-19 was declared a worldwide pandemic on 30 January 2020 by WHO. Until the WHO declaration, the global impacts of COVID-19 were only speculated upon, and many nations were unprepared to combat the pandemic. The government's decision on the management of the COVID-19 crisis and the variation in the strategic applications of cognizing, communicating, coordinating, and controlling became a predominant approach in facing the pandemic globally (Parmeshwar et al., 2020). Globally, various legislative approaches were exercised in minimizing public health vulnerability to pandemic exposure in which emergency laws were enacted for imposing lockdowns in nations and restricting domestic and international flights and travel, curfews, and limiting the available services, aimed to stop COVID-19 rise at the community level. However, the strict restrictions imposed on international trade transportation of raw materials aggravated the adverse impacts on Production as well as trade from local to global (FAO, 2003, 2020a, b; Laborde, 2020). The emigration of people from high labor-demanding areas increased the availability of labor for agricultural and allied activities. However, the increasing availability of laborers in agriculture for local or unskilled employment would have restricted their income, which was highly dependent on natural resources to generate low-value products. On the other hand, the areas intensively depend on agricultural labor demand for agricultural activities, livestock management, marketing, and creating agricultural infrastructure encountered labor shortages. The potential outcome noticeably affected the global food supply. The aggravating impacts of COVID-19 on the supply of food and associated food security components made the countries procure and protect stock and restrict international trade. Therefore, COVID-19 has been observed as an unprecedented public health crisis that significantly resulted in an economic slowdown, threatened food production, and potentially resulted in global supply threats. These effects and restrictions imposed on trade in major producing countries might have significant consequences for food security in trade-dependent nations. These effects could deteriorate global supply chains in 2020 and beyond. The potential threats to Production and restrictions on trade due to COVID-19 have been observed to decrease the supply of food in import-dependent, and vulnerable countries, which eventually affected international food trade (Parmeshwar et al., 2020).

COVID-19 Implications on Global Cereal Supply

The major exporting countries comprise approximately 70% of the cereal export and 45% of its Production worldwide. The USA is the largest cereal exporter, which accounts for nearly 16 and 18% of global Production and export, respectively. China

ranks among the top 10 cereal importers worldwide and is the largest cereal-producing country, accounting for 21% of Production globally. Production threats and restrictions on global food trade have significantly proved the impacts on importing countries. In 2020, COVID-19 caused a 24.8% decrease in the exportation of agricultural food products (Laborde et al., 2020). COVID-19 cases were observed to increase continuously, due to which the span of restrictions on trade also increased. Nearly about more than 2 million cases of COVID-19 were reported on the 5th of May, 2020, and approximately 29 countries restrict food trade, resulting in an approximately 5% decrease in food markets globally (IFRI, 2020; Laborde et al., 2020). Different countries-imposed restrictions on food trade, e.g., various food exports were banned by Eurasian Economic Unions, and new rice export contracts were suspended by Indian traders. Rice export permissions were suspended by Myanmar, Vietnam restricted the export of rice, the Russian Federation restricted the export of wheat and other cereals, Ukraine restricted the export of wheat, Sudan imposed a ban on the export of maize, sorghum, and various other countries have restricted trade, only to secure their supplies at a domestic level during and after the COVID-19 pandemic (ITC, 2020; Wertheim-Heck et al., 2015).

COVID-19 Implications on SDG-2 (Zero Hunger Goal)

The accessible sufficient quantities of sustainable food products is a key to achieving SDG-2. In 2019, the United Nations Economic and Social Council report on SDG-2 progress raised concern with regard to the increasing number of insufficiently nourished population from 784 million in 2015 to 821 million in 2019 and decreased government expenditures on agricultural aid. COVID-19 impacts on food security (Fig. 6.1) were observed to persist beyond 2020; this wave is still noticeable in the present era (UNESC, 2019).

The 2020 Global Report on Food Crises (GRFC) has reported that in more than 50 countries and territories, nearly 135 million people experienced acute food insecurity before the crises of COVID-19 (FSIN, 2020). One of the significant responses of worldwide countries to the health crisis caused by the pandemic was prioritizing ensuring their food supply (ITC, 2020). Covid-19 threatened global food security but immensely affected the countries dependent on food imports that were found to be most vulnerable to the trade restriction impacts. FAO proclaimed an advisory for the countries in order to resist import-export restrictions against this threat (FAO-Agri, 2020a; FAO-Coronavirus, 2020b). In developing countries, the cereal composition of food is estimated to be 159 kg/person/year (global average160), and cereals for all consumers are estimated to be 254 by 2030 (Alexandratos & Bruinsma, 2012).

However, developing countries are the most vulnerable with a cereal supply of about 38 to 153 kg/person/year at a domestic level. In sub-Saharan Africa, the hunger situation was observed as rigorously alarming in the Central African Republic,

Fig. 6.1 Potential factors affecting global food security. (Parmeshwar et al., 2020)

Chad, Yemen, Zambia, Madagascar, and Liberia in accordance with the World Hunger Index 2019 (Gopalan & Misra, 2020). While South and South East Asian countries, India, Pakistan, Afghanistan, Indonesia, Philippines, and Colombia had suffered severe hunger shocks. Most of the population in these countries relies on agriculture and daily-waged activities (Global Hunger Index, 2019). The COVID-19 economic slowdown in these countries, with increasing unemployment, decreasing income, and supply chain disruptions, could severely jeopardize food safety in poor societies. The pandemic is mainly causing food insecurity in developing countries. Potentially decreasing food production, depleting reservoirs, and trade restrictions have likely been observed to adversely affect food safety and security in 2020 and 2021 (Sumner et al., 2020).

The pandemic impact on food security may persist longer through slowing down of the economy, decreasing agricultural investments, government expenditures, and aiding farmers. The COVID-19 is expected to increase uneven poverty worldwide, adversely affect the progress made since the decades on diminishing poverty, and thereby affect progress towards the better achieving of SDG-2.

Food Safety in Developing Countries

The food market is in line with other markets regarding quality and safety attributes that are not observed. Most food safety hazard tests are expensive in terms of the food value, making examining food at each transaction nearly impossible. Therefore, food sellers and consumers need to be made aware of the food safety and security rules. Inappropriate information between sellers and consumers can cause market failure where food quality is not appropriately examined, adversely affecting agricultural and pharmaceutical sectors in developing countries (Björkman et al., 2013; Bold et al., 2017).

The asymmetric information with regard to food safety implies that public intervention through enforcing regulations against unsafe food buying and selling can enormously improve aggregate welfare. Cost analysis and positive impacts of combating foodborne illness in developing countries are scarcely sufficient, but presently analysis of high-level has estimated that higher investment is necessary (Jafee et al., 2018). A significant barrier to improving food safety in developing countries has been observed due to public institutions often confronting resource and capacity restrictions that reduce their effectiveness. The World Organisation for Animal Health has evaluated that out of 35 assessed developing countries, only two were adequate to identify and trace animal products, and around 6 developing countries could sufficiently inspect facilities regarding meat distribution, strict rules and regulations for veterinary drugs, and capable of ensuring the quality of laboratory where animal products are tested (Jafee et al., 2018). Moreover, awareness of food safety hazards and steps to mitigate such risks are being observed to be lesser where attainment of educational levels has been found low. In developing countries, less income and fewer awareness levels equally make a customer less voluntary to pay for food safety. Eventually, food security and safety should be prioritized by the government or markets (Humphrey, 2017).

In developing countries, the food safety testing costs are exceptionally high with regard to food transaction value because of the availability of smaller firms in the food sector, consumption of food products of low value, and smaller transaction volumes. There needs to be more systematic information on food safety testing costs. Equipment costs and consumables of the laboratory may be higher in relation to less demand in the market for the products due to increased scale of distribution and import duties. Although less information on both sides of the market can only be resolved by decreasing the costs of food safety testing. Unsymmetric information can be resolved if well-aware consumers demand a product of high quality.

In developing countries, the low willingness of a consumer for paying, as well as the deficit in auditing firms of food safety and deficient independent food testing laboratories, reduce the value of combating market failure caused by unsymmetric information. Moreover, food markets in developing countries are generally governed by the informal sector, operating outside of the control of regulations. In the formal food sector, the market share tends to remain at its minimum, particularly for fresh food commodities (Attrey, 2017; Frayne et al., 2010; Grace et al. 2015;

Manning & Soon, 2016; Tangendjaja et al., 2016). Strict regulations and high taxes in developing countries are enforced in the formal sector, thereby reducing the incentives of firms, which suggests that the attempts to improve food safety and security by the enforcement of regulations could have outcomes that are unintentional so as to decelerate the formalization in the agricultural food sector (Fajnzylber et al., 2011).

Pesticide Contamination in Food and Its Control

The widespread problem of pesticide-contaminated food products in India is considered extremely difficult to rectify. Many factors are highly responsible for pesticide penetration in food commodities. Since India is a country of agriculturalists, where a major population is employed in the agriculture sector (Singh et al., 2013), this basic characteristic makes India vulnerable to pesticides (Abhilash & Singh, 2009). Indian farmers are mostly less aware of using pesticides in a proper way and the time of harvesting (Kumari, 2008). It causes premature harvesting, and therefore, many pesticide residues in crops are found. Despite being banned from marketing, pesticides freely available in the market are toxic in nature, which vehemently violates public safety laws. Many research scientists and individual organizations have tested such pesticide residues in Indian food products and submitted their reports to higher authorities (Pronczukd, 2008). In spite of this, negligible strict measures have been taken for prevention. India needs an advanced approach to keep pesticides from further contaminating food products. Pesticide-causing diseases occur at an increasing rate in developing countries compared to developed countries. One of the major causes of this incongruity is the need for more clarity in transferring regulatory information from the countries that export pesticides to the pesticide-importing countries (Ecobichon, 2001).

The advanced technologies in the countries might make them the leaders in developing and registering pesticides and disseminating information to less sophisticated stakeholders. Furthermore, pesticide users in importing countries need appropriate training and education to handle, apply, and store pesticides. A standard regulatory system would effectively regulate pesticides worldwide. However, an international regulatory system that binds all countries may only be applicable later. Regional organizations contribute by offering a successful model to develop a single regional unit for promoting the safe and positive use of pesticides. Registration of pesticides at the regional level and regulation activities would improve the agricultural product trade and keep the environment from pesticide hazards.

The FAO developed the International Code of Conduct on Distribution and Use of Pesticides in collaboration with the WHO, International Labor Organization (ILO), United Nations Environment Programme (UNEP), International Group of National Associations of Manufacturers of Agrochemical Products (GIFAP) (Donovan et al., 2001). The main purpose of this code was to harmonize pesticide-exporting and importing countries. Various banned pesticides were exported to

developing countries prior to this code, which is deficient in technical, legal, and administrative resources to have access to pesticide toxicity. The code provides a Prior Informed Consent (PIC) portion updating the country with information on pesticides that are imported (Kesavachandran et al., 2009). The code is, however, intended and legally unbound. It was set up to act as a temporary measure until local governments developed authenticated rules and regulations (Donovan et al., 2001).

One of the critical initiatives for the management of pesticides is the FAO/WHO Joint Meeting on Pesticide Management (JMPM) and the Annual Session of the FAO Panel of Experts on Pesticide Management. The JMPM was set up to harmonize FAO and WHO for adequate pesticide management. The annual meeting encountered FAO and WHO with the regulation and pesticide management and made them implement the International Code of Conduct on Pesticide Management (FAO/WHO, 2010, 2013). The Codex Pesticides Residues in Food Online Database includes the maximum residue limits (MRL) and extraneous maximum residue limits (EMRL) for the adoption of pesticides by the CAC (Codex, 2013). Globally, different authenticated organizations and scientific databases address various issues regarding the use of pesticides, their health effects, and environmental shocks. Codes implemented on the basis of databases and MRL potentially curtail the harmful effects of pesticides. Kumari (2008) found that washing and blanching vegetables lead to around 22–60% reduction in pesticide residues. While Peeling leads to a 70–100% reduction in pesticide residues. The washing and boiling of the vegetables affect different factors of pesticide residues (Fig. 6.2) (Kumari, 2008).

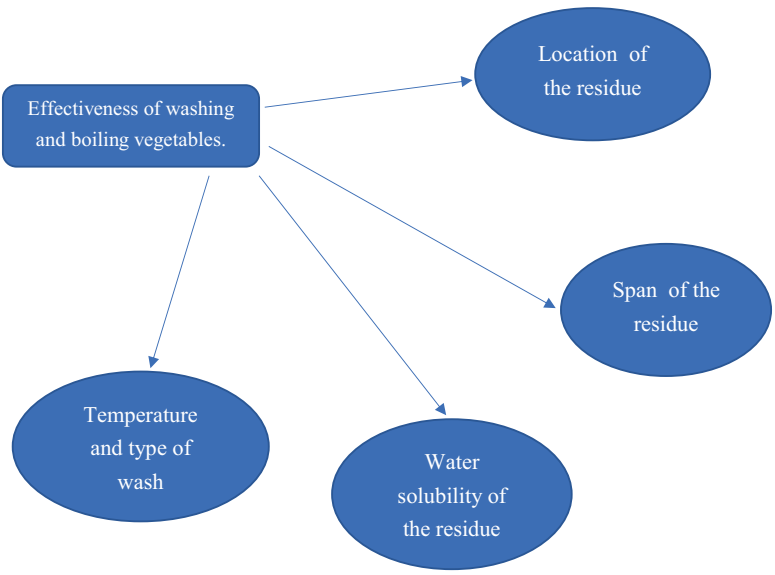

Fig. 6.2 Effectiveness of washing and boiling vegetables on the different factors of pesticide residue. (Kumari, 2008)

Good Agricultural Practices for Pesticide Residue Management

(i) Keep a record of chemicals. Store all chemicals in their respective containers. Do not store herbicides along with other pesticides.
(ii) Only allowable pesticides with recommended dosage and frequency should be used at an appropriate time. Banned pesticides should not be used.
(iii) Education and training should be given to use pesticides properly. The lack of knowledge can lead to residue problems.
(iv) Dispose of unused pesticide solutions which are generated by cleaning spray pumps to avoid pollution since they contain pesticide residue.
(v) Indiscriminate use of pesticides should be avoided.
(vi) Follow an Integrated Pest Management System.
(vii) Religiously follow the waiting period before the time of harvest.
(viii) Prepare healthy soil with compost and mulch in order to reduce pest effects.
(ix) Fruits and vegetables should be thoroughly washed with tap water.
(x) Decrease the drifting of spray in orchards by reducing pressure, using nozzles with larger diameters, and pesticides of a less volatile nature.
(xi) Read the labels of the product carefully before applying and mixing any pesticide as mentioned (Shailesh et al., 2013).

Women: One of the Important Pillars of Food Safety and Security

The first pillar of food safety and security is the Production of food in a sustainable manner. In sub-Saharan Africa, women comprise nearly 70–80% of household production of food, whereas, in Asia and Latin America, they account for 65% and 45% of food production in households, despite unequal access to inputs, land, and information. Women farmers can receive equal or higher yields than men farmers if they are provided equal accessible resources and human capital. Laws governing their rights to land vary widely. In sub-Saharan Africa, where women are prominently responsible for food production, their rights to use land are generally limited. Some irrigation projects have limited their rights to land (Battersby & Crush, 2014). Despite their prominent Role in agriculture, women are denied an appropriate agricultural extension share and other services. During COVID-19, food insecurity among women was approximately 10% higher than among men and even more so in developing countries (FAO/WHO, 2018). Had women in rural areas the same exposure to productive activities as men, agricultural Production and farming would have been increased, and nearly 150 million more human population could have been fed.

FAO states that in developing countries, women account for 43% of the agricultural workforce. Food safety is not just attributed to food availability, nor to be accessed by financial resources. People must also have accessible quality and

nutritious food to ensure food security. Various types of research show that gender inequalities threaten the ability to sustain food and nutritional food security (Carmen, 2017). One potential remedy is to give agricultural training to women and increase their number as agricultural extension agents. This practice would rip out the cultural restrictions against the extension of male folk and the interaction of female farmers. It would also enable women to share information among themselves in the groups. Agricultural research institutes also need to implement their indigenous knowledge in farming systems. Educating women about basic agricultural practices would increase agricultural productivity and income (Carmen, 2017).

The second pillar of food safety is access to available food economically. Various studies have found that improvements in household welfare depend on who earns along with the level of household income. It has been found that women are likely to spend their income out of proportion on food for their family members. Moreover, improvements in the health of children and nutrition status are more strongly attributed to women's incomes. The third pillar is to achieve nutrition security which includes adequate energy, protein, minerals, and micronutrients for the family and also depends on an adequate quantity of household food and factors, including child health care and accessible purified water and sanitation (Carmen, 2017).

The exclusive sphere of activity of women is to ensure the nutrition security of the household via food and other resources. Protecting the nutrition status of females is vital to provide a head start for the nutrition status of children. Improving the pre-pregnancy status of nutrition, increase in weight during pregnancy, lactation diet, Production of breastmilk, and better-nourished mothers increase birth weight in infants and improve children's health. However, women's nutrition status is endangered when they are treated as shock absorbers for the household by the liquidity of the status of their nutrition in meagre seasons. Moreover, it has been found that in South Asia, a strong pro-male and pro-adult bias within the family in distributing food and other resources also tends to deteriorate the health of women and their status of nutrition.

Conclusions

The demand for food products is increased at higher risk of contamination, including animal food and fresh produce, and there exists limited government for identifying outbreaks, implying that force on the food security and safety system is growing unabatedly. Consumer demand for food safety has been estimated by various research studies. The government possesses limited capacity for enforcing the regulations, and independent food safety and testing laboratories are rare. Surveillance of food is used for investigating and potentially controlling contaminated products in food. Various food laws have been implemented to ensure food safety worldwide. The hunger problem worldwide cannot be resolved only by improving productivity but also resolved by the developed science-intensive technologies that favor manufactured product preservation, the development of various logistic schemes, and

agricultural Production. Ensuring food safety and supply needs official policies and legislative measures to provide a unified national action and strategies framework.

COVID-19 has led to severe and unprecedented disruptions in local as well as global supply of food. However, major cereal producers show an increment in the supply of cereals domestically. COVID-19 led to restrictions on trade and prices that eventually affected agricultural income and GDP. Various restrictions and regulatory activities have been imposed worldwide to control the pandemic situation. Recently vaccination has also been done to combat the pandemic. However, particular attention should be paid to ensuring the safety and security of food in the present era and beyond. Persist initiatives assisting purchasing power of households and enhancing the options of trade should be prioritized in different government policies.

The use of pesticides is growing worldwide, and various countries are looking for different ways in order to educate consumers about the advantages of chemical pesticides without being threatened by their application. Various countries often import required chemicals to increase the Production of home food and export different yields of crops. Due to deficient infrastructure and forces, some countries are clinging to various information, including labeling, using patterns, application rates, material safety data sheets, etc., provided by international manufacturing agencies. There are international codes of conduct for promoting cooperative efforts and shared responsibilities among parties. Many countries also have specific rules and regulations for the utilization of pesticides. Pest management is one of the significant inputs in the Production of agriculture. Therefore, this area grabs great attention to economizing Production, providing safe foods, and lowering medical expenses for combating various ailments. In addition, alternative measures for pest management should also be explored to consume, Import, and export safe products in the future.

National and international organizations must take crucial steps to permit women to accomplish their potential in the generation of food safety and security. They need to enhance the physical and human capital of women. The ability of women in the Production of food can be increased by enhancing their access to resources, information, and technology. Educating and training women would increase productivity in the present and future eras.

In conclusion, food safety and nutrition are connected by a close nexus. Unsafe food leads to various diseases and malnutrition among the living population, as the food supply chain crosses many national and regional borders. The collaboration between the government, producer, supplier, distributor, and consumer would eventually ensure food safety in the present era. The introduction of the FSSA could boost domestic and international consumer confidence and make the nation meet international food security standards. However, its implementation yet faces various challenges. There is still a lack of general awareness of the hazards that are associated with unsafe food practices, and thus the safety procedures and methodologies are yet to be followed. The poor infrastructure of testing laboratories, insufficient technical expertise, and skilled manpower for legislating the rules are still of great concern. Problems in tracing the products from the manufacturing to the processing unit are one of the implementation challenges of FSSAI in the present era. The

NGOs can spread awareness publicly about food contamination and the use of safe food. Thus, these organizations can act as an essential connection between the government and the people and direct the masses in various activities to protect the environment.

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Chapter 7
Roles of Biotechnology in Environmental Monitoring in the Food Industry

Nurul Hawa Ahmad

Introduction

Biotechnology is a massive discipline and can be crosslinked to any scientific field. The term 'bio' refers to the utilization of living organisms (e.g., plants, microbes) or components derived from living organisms (e.g., genetic material, enzymes, bacteriocins). In the food industry, biotechnology is often correlated to the invention of new products or new ingredients using living organisms, such as genetically modified (GM) crops, to achieve food and nutrient security. Another aspect of biotechnology is the development of cutting-edge detection and risk-based approaches as control measures against microbial and allergen contaminants.

Many foodborne pathogens isolated from the food environment have been identified as causative agents for major outbreaks and recalls. In Finland, 13 out of 687 *Listeria monocytogenes* strains isolated from 2015 to 2021 during outbreak investigations were sourced from a food processing environment (Suominen et al., 2023). Cold-smoked salmon recalls in the U.S. have been associated with inadequate sanitation controls, as FDA inspections found 13 out of 15 salmon processing facilities were detected with *Listeria monocytogenes* (Cripe & Lasikoff, 2021). Infant formula manufactured in Michigan, USA, was tainted with *Cronobacter sakazakii*, causing nationwide and international recalls, eventually leading to global shortage between 2021 and 2022 during the COVID-19 pandemic (FDA, 2022a).

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Scope of Environmental Monitoring (EM) in the Food Industry

Environmental monitoring (EM) in the food industry covers food contact surfaces, nonfood contact surfaces, and personnel. The primary goal of EM is to avoid contamination of the finished products. Finding sources of microbial contaminants should be the priority as pathogen distribution in the food processing environment is heterogeneous. Therefore, all levels of employees should have knowledge of EM in the food industry to help them handle present and emerging food safety risks.

Food Contact Surfaces

Food contact surfaces are surfaces that may encounter food or food drainage during production, processing, and packaging. Food contact surfaces should be manufactured, designed, and operated in a hygienic manner to avert the harborage of microorganisms (Skåra & Rosnes, 2016). Stainless steel, plastic, rubber, wood, glass, and ceramic are common materials used to construct food contact surfaces. Food contact surfaces should be smooth, non-corrosive, durable, and not easily scaled, scratched, distorted, or decomposed (FSIS, 2016). Food contact surfaces should be cleaned easily, do not impart color, and allow the migration of harmful substances. Well-designed equipment should be free from sharp internal angles, corners, and crevices, as well as uneven welds and joints. Examples of food contact surfaces include utensils, conveyors, slicers, mixing tanks, and packaging materials. The degree of desirable attributes for single-use food contact surfaces may slightly differ from those for multi-use food contact surfaces.

Nonfood Contact Surfaces

Nonfood contact surfaces are surfaces that may indirectly come into contact with food. Nonfood contact surfaces should not collect debris, dirt, and food residue. Several examples of nonfood contact surfaces include buttons of machines, doors, stairs, floors, carts, drains, ceilings, windows, walls, and pipelines. EM on nonfood-contact surfaces should be prudently conducted because if overlooked, microbial contamination on nonfood-contact surfaces may reach finished food products over time.

Personnel

Essentially, all people who are involved in any food contact surfaces and food packaging materials must be equipped with hygienic practices. Maintaining cleanliness, wearing appropriate attire, and controlling disease make up standard hygienic practices that cannot be compromised at any time (FSIS, 2016). Cross-contamination by workers via contact with personal items, human body parts, and human discharge can transmit foodborne pathogens to the finished products. For instance, food workers who directly handle cooked foods may be touching nonfood contact surfaces such as wheeled carts. If workers are not stationed in a single processing area, the likelihood of spreading the illness is much greater. The health status of workers should also be monitored, and proper attire, including hairnet, shoes, and gloves must be worn. Individuals who handle food and operate food processing equipment are required to sit for food safety training. Training is required to understand the basic food safety principles and hygienic practices to avoid unintentional cross-contamination (FDA, 2022b).

Environmental Monitoring Program (EMP)

To put it into context, it is pertinent to see where EMP lies within the food safety management system, a comprehensive and internationally recognized procedure to safeguard food safety. A basic food safety management system consists of a Hazard Analysis Critical Control Point (HACCP), Good Manufacturing Practice (GMP), and prerequisite programs (PRPs). HACCP is the top of the food safety hierarchy, followed by PRPs and GMPs, which serve as the backbones of the HACCP foundation. HACCP is a science-based approach to reduce or eliminate food safety hazards at critical control points (CCPs) of food processing (FDA, 2022c). On the other hand, GMPs and PRPs are day-to-day protocols to monitor operational conditions so that CCPs can effectively reduce or eliminate food safety hazards. In the last decade, food safety management system has evolved to adopt customer-driven standards, including Safety Quality Food Institute (SQF; first edition in the 1990s), British Retail Consortium (BRC; first edition established in 1998) or Food Safety System Certification 22,000 (FSSC 22000; first edition established in 2009) (Moerman & Wouters, 2016). Lee et al. (2023) found that food safety culture and management leadership were positively increased in small and medium food manufacturers in selected developed and developing countries after implementing the food safety management system.

EMP is one of the prerequisite programs (PRPs). Although the principle of EMP is generic across the food industry, the establishment of EMP is largely dictated by the food group, intended consumer, and operational facility. For instance, a baby food processing plant may not apply the same EMP protocols as a frozen vegetable processing plant. EMP is critical for the food industry, which (1) produces foods

that involve the pathogen inactivation step, (2) produces RTE products that are exposed to the environment after the pathogen inactivation step and prior to packaging, (3) produces RTE products that do not involve pathogen inactivation step, and (4) produce foods that involve chilled ingredients or products that can support growth of *Listeria monocytogenes.* EMP should be established for a new food processing facility and partially/wholly renovated food processing facilities (Holah, 2014).

To establish an effective EMP, food manufacturers must ensure that processes and procedures for maintaining infrastructure, operating sanitation and pest control programs, and inspecting raw materials are running in the ideal state. Following that, food manufacturers must establish a written plan that sensibly reflects all EM components within their facilities. Each EM component must be thoroughly considered. A good EMP plan should include specific corrective actions in which uncontrol situations can be corrected so that hazards can be stopped before reaching the noncompliance limit.

Hygienic Zones

EMP requires that hygienic zones of the food processing plant are clearly indicated and laid out. Compartmentalizing hygienic zones in a food processing facility is critical to avoid cross-contamination within the facility. The layout of the hygienic zone should be from raw to clean, wet to dry, and unprocessed to the processed zone. Movement of wheeled equipment and employees must be controlled as these are some routes that can introduce contamination sources. Hygienic zones also dictate the frequency of sampling. Four categories of hygienic zones are:

- Zone 1 – direct contact: Product contact surfaces
- Zone 2 – indirect contact: nonproduct contact
- Zone 3 – more remote nonfood contact
- Zone 4 – nonfood contact surfaces outside of the processing

Impact of Noncompliance

Negligence of EMP can have a serious impact on the food industry. Factors contributing to EMP noncompliance include the absence of a corrective action plan, dated sampling strategy, archaic food processing layout and operation conditions, disordered record keeping, and outdated microbiological safety knowledge. Without a proper corrective action plan, food products that are improperly pasteurized or processed may be released to the market, posing a great risk of product recalls and foodborne outbreaks. A combination of a dated sampling strategy and outdated food processing layout may yield false negative results because niche areas of food

contact surfaces or indirect surfaces are not tested. Outdated microbiological food safety knowledge in many levels of food workers may cause the emergence or re-emergence of pathogens to remain undetected. Eventually, long-term implications such as monetary loss, lawsuits, and destroyed brand reputation can sink food operations and never recover.

In many cases, consequences of noncompliance can induce positive transformation in the food industry because food manufacturers are going above and beyond to focus on resolving authoritative issues. Food operations may be stopped entirely to scrutinize factors that can lead to non-conformance. Monetary resources and organizational support are instantly available to deal with foodborne outbreaks and product recalls (Armentrout, 2022). Once issues are sorted out, self-inspection and self-monitoring should be carried out continuously for overall safety and quality improvement. Moving forward, food manufacturers may be more alert, conscious, and stringent measures for ensuring food safety and quality. Elevating knowledge is critical for behavioral change. Therefore, all levels of workers should be nurtured and trained according to current food safety regulations.

Roles of Biotechnology in Monitoring the Food Environment

Detecting Potential Hazards

Food processing environments pose high nutrients and moisture, which are favorable for microbial growth. There are four main routes for potential contaminants to gain access to food processing areas: (1) raw materials, (2) external environment, (3) sick food workers and facility visitors, and (4) pathogen testing laboratories (Holah, 2014). The likelihood for potential contaminants to transcend into the food processing area depends on factors such as types of food produced, severity of hazard, and infrastructure layout.

The food industry is the arena in which raw materials turn into ready-to-use ingredients or ready-to-eat food products (Dadhaneeya et al., 2023). Inspection of raw ingredients can be the first line of defense to stop microbial contamination from entering the food processing areas. Visitors (e.g., contractors, site auditors, regulatory inspectors) can introduce contaminants in the food processing area.

Types of Microbial Contaminants

Indicator Microorganisms

Indicator microorganisms reflect food, water, and environmental quality and hygiene. The presence of indicator microorganisms can tell us whether there is a potential presence of pathogens, process failure, or inefficient sanitation protocols.

In general, the detection of indicator microorganisms in the food industry involves the presence of aerobic plate count, *Enterobacteriaceae,* coliform, and fecal coliform.

Aerobic plate count (APC) also known as total plate counts (TPC), measures total microorganisms that grow best in the presence of oxygen and at a temperature of 35 °C. *Enterobacteriaceae* is a wide family of microorganisms that includes members of coliform groups (*Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Hafnia*, *Serratia*), *Shigella*, *Yersinia*, and *Salmonella*. *Enterobacteriaceae* are able to ferment glucose, whereas coliforms are capable of fermenting lactose, with the production of gas and acid at 35 °C. Fecal coliform is a subgroup of coliform that ferments lactose at a slightly higher temperature, ~ 45 °C. Fecal coliform is the best indicator of the presence of *Escherichia coli* (Eden, 2014). Other than coliform, *Pseudomonas* spp. may be tested to signify postprocessing contamination, particularly in milk products. *Pseudomonas* spp. is not a member of the *Enterobacteriaceae;* therefore, the presence of *Pseudomonas* spp. may go undetected using standard environmental monitoring protocol in the food industry (Rojas et al., 2020) (Table 7.1).

Table 7.1 Selected rapid/alternative methods for detecting indicator microorganisms that can be used in the food industry

Rapid/Alternative methods	Principle	Microbiological test	Samples	References
TEMPO system	Semiautomated MPN technique with two main features: A card containing multiple sets of tenfold serial dilutions media that mimics MPN experimental design. Fluorescence reading to observe growth	APC Coliform	Milk products	Lindemann et al. (2016)
Lateral flow test strip (LFTS) using colloidal palladium nanoparticles (PdNPs) and HRP	Enhanced sensitivity of immunoassay technique that Uses Abs-labeled PdNPs rather than colloidal gold nanoparticles Oxidizes DAB as a colorimetric signal	Coliform	Meat and poultry	Tominaga (2019)
Non-lytic *M13* phage	Bacteriophage biosensor	Coliform	Water	Sedki et al. (2020)
Graphene-Polyacrylamide gel dual substrate sensor platform	Biosensor (optical and electrochemical)	Coliform	Nonspecific	Badalyan et al. (2018)

APC Aerobic Plate Count, *MPN* most-probable-number, *Abs* antibodies, *HRP* horseradish peroxidase, *DAB* 3,3′ – diaminobenzidine

Index Microorganisms

The presence of index microorganisms may indicate the likelihood of pathogens of concern in food or environment environments. For instance, *E. coli* can be categorized as pathogenic and nonpathogenic. Because *E. coli* is commonly found in mammalian feces, the presence of *E. coli* is utilized to assess water quality. Index microorganisms can also reflect post-processing contamination. *Listeria* spp. Detection is used to monitor the presence of *Listeria monocytogenes* in smoked salmon, deli meat, ice cream, and cheese processing plants, as *Listeria* spp. can proliferate in a cold environment. The selection of index microorganisms also depends on the food industry. Infant formula processing plants may monitor *Salmonella* spp. and *Cronobacter sakazakii* detection as these pathogens are known to be thermally resistant strains.

Verifying Cleanliness and Sanitation

The food processing step is not complete without performing cleaning and sanitizing at the endpoint of daily operation. There is a clear distinction between cleaning and sanitizing. The purpose of cleaning is to remove food debris and organic matter on food contact surfaces. Detergent is used to perform cleaning on food contact surfaces. The purpose of sanitizing is to kill microorganisms by applying antimicrobial constituents. Disinfectant is used to sanitize food contact surfaces. Cleaning must precede the sanitizing step because dirt and other materials on the food contact surface impede the antimicrobial constituent to be effective. In most cases, an effective cleaning protocol is able to remove 90–95% of microorganisms.

Biofilm is an accumulation of bacterial cells within extracellular polymeric substances (EPS) that adhere to surfaces over a long period of time. Biofilm formation, particularly on niche surfaces of food processing equipment, remains a significant hurdle in the food industry. Wet food environments such as meat, poultry, seafood, fruits, and vegetable processing plants are prone to harbor biofilm formation because these areas are rich in nutrients and contain high microbial load. Eradication of biofilm is critical because many foodborne outbreaks have been associated with biofilm, including *Listeria monocytogenes* and *Salmonella* spp. (Dallagi et al. 2023).

Methods of Cleaning and Sanitizing

Clean-in-place (CIP) is specifically used for cleaning and sanitizing bounded interior parts of processing equipment, including heat exchangers, vessels, pipes, tanks, and fillers. CIP can be carried out automatically via verified programmed cycles to deliver optimum efficiency. CIP offers convenience for huge and continuous food systems. Hygienic design is critical for CIP application, which ensures the circulation of cleaning and sanitizing solutions is able to reach all interiors of the equipment (FSIS, 2016).

On the other hand, clean-out-of-place (COP) is useful for batch processing equipment, which can be easily disassembled for cleaning and easy to assemble for use. COP is more labor-intensive and time-consuming, as compared to CIP, but COP can reach niche areas that can harbor microorganisms. To perform COP, manual cleaning such as wiping, scraping, brushing, and scrubbing is sometimes needed, but foaming and high-pressure methods can aid the cleaning process, in removing grease or protein layer that is difficult to remove by hand-scrubbing. To determine cleaning protocol accuracy, Losito et al. (2017) proposed compliance criteria of good hygienic conditions of compliant (from not detectable to 49 CFU/cm^2), improvable (between 50 and 499 CFU/cm^2), and not compliant (> 500 CFU/cm^2).

Allergens Monitoring

Allergens are proteins that can cause adverse immunological responses. Allergens monitoring is critical in the food industry because there is no cure for food allergies up till now. Food allergies can cause anaphylaxis and even death. Allergen labeling has been enforced to protect susceptible consumers; however, the information provided can be misleading due to inconsistent and ambiguous labeling format (Zhu et al., 2022). World Health Organization and International Union of Immunological Societies (WHO/IUIS) database has listed allergenic proteins derived from plants (509), animals (465), fungi (120), and bacteria (1) to date (WHO/IUIS, 2023). Nevertheless, major food ingredients declared as food allergen in the United States and European Union are as follow:

- European Union: Milk, fish, eggs, crustaceans, mollusks, nuts, peanut, soybeans, cereal containing gluten, sesame, celery, mustard, lupin, sulphur dioxide, and sulphites >10 mg/kg or 10 mg/L (FSA, 2015)
- United States: Milk, fish, eggs, crustacean shellfish, tree nuts, peanut, soybeans, wheat, sesame (FDA, 2023)

Given that many undeclared allergen cases were caused by accidental cross-contamination (Martínez-Pineda & Yagüe-Ruiz, 2022), a risk-based approach requires food processing facilities to establish a food safety plan for allergen monitoring. One efficient way to prevent allergen cross-contacts is by using dedicated processing equipment for a single allergen. Due to space constraints and production costs, many food manufacturers are using the same equipment for multiple allergens or food without allergens. After cleaning and sanitizing processing equipment, the presence of allergen residue must be tested.

Conventional methods of detecting allergens, such as Enzyme-linked immunoassay (ELISA), real-time PCR, and mass spectrometry such as HPLC or LC-MS/MS, have been used as rapid test kits with reasonable sensitivity. Several limitations should be addressed, including cross-reactivity with nontarget food components or denaturation of proteins during food processing. These limitations make it difficult to trace allergen residues in thermally treated, hydrolyzed, and fermented foods, which may lead to false positive/negative results.

Table 7.2 Aptamer-based methods for allergen detection

Detection method	Principle	Specific target	Samples	References
Plasmonic genosensor	Measure real-time refractive index changes when the target interacts with the surface of the SPR biosensor. Label-free analyte	2S albumin – Coa a 14 gene	Hazelnut	Moreira et al. (2023)
Aptamer-modified carbon dots	Fluorometric sandwich biosensor Employ MIP and aptamer carbon dots as recognition to improve selectivity toward the target	Tropomyosin	Seafood products	Wang et al. (2022)
Mesoporous aptasensor	Fluorescence biosensor Uses fluorescent dye rhodamine B loaded in nanoporous anodic alumina support O1 conjugated with O2-O4 used as aptamers.	Giadin (gluten)	Wheat products	Pla et al. (2021)

ELISA enzyme-linked immunosorbent assay, *O* oligonucleotides

On top of a long list of allergenic proteins based on the WHO/IUIS database and the possibility of false negative/positive results, researchers have developed aptamer-based detection methods to improve reliability for detecting allergens. Aptamers are single-stranded oligonucleotides and have many advantages over traditional antibodies. Aptamers are easier to synthesize, pose higher affinity and specificity, are cheaper, and are not easily denatured when exposed to high temperatures (Kaur et al., 2018). Several aptamer-based biosensor methods for allergen detection are listed in Table 7.2

Removing Pollutants from Wastewater

Our world has limited water resources, and it is predicted that the water deficit will intensify by up to 40% by 2030 if no preventive measures are taken in a global scale (OECD, 2012). In this decade, the rapid effect of climate change has forced more stringent policies to be developed nationally or regionally to improve water security (Hejazi et al., 2023). Water is a valuable resource for the food industry because water is used for washing, cleaning, blanching, sterilizing, and many more. Water must be managed to achieve its optimum use for sustainable food production. Water generated from any food processing step should be reused, if possible, or treated to protect the environment.

Wastewater from the food industry carries high amounts of nutrients, suspended particles, and organic substances, which need to be removed before being released to local sewage (Saravanan et al., 2022). Biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), and nutrients such

as nitrogen (N), and phosphorous (P), are among the measured parameters to determine water quality. Biochemical oxygen demand (BOD) refers to the O_2 requirement to break down organic material in the presence of microbes. COD refers to the O_2 requirement to break down organic material (including non-degradable substances not measured in BOD). Good quality water should pose low biological and chemical indicators, expressed in mg/L.

The biological and chemical composition of wastewater depends on the type of food industry. High fat, oil, and grease levels are commonly generated by vegetable oil and meat and poultry processing facilities. Carbohydrate-rich wastewater is typically discharged by beverages, breweries, and the cane sugar industry. Wastewater with high protein levels comes from the industry manufacturing dairy and fish products. Oil and lubricant from food processing equipment are also discharged in wastewater (Srivastava et al., 2022). Processing facilities must use appropriate treatment methods to ensure waste components are reduced to acceptable BOD and COD levels.

Anaerobic digestion is a biological method used to treat wastewater. Anaerobic digestion can reduce about 90% of BOD levels via the action of the microbial community. Anaerobic digestion involves three main stages: hydrolysis, acidification, and methanogenesis. During hydrolysis, complex organic matter is broken down into simple compounds. Later, acidification occurs when the mixture undergoes fermentation, converting simple compounds into acetic and volatile fatty acids. The last stage, methanogenesis, of results from methanogens converting acetic acids to methane. A thorough explanation of anaerobic digestion in wastewater is well-described by Saravanan et al. (2022).

Wastewater also carries pathogens. Reusing water from food industry wastewater can be a concern, especially for direct use. Water aimed for drinking should be free from coliform and *E. coli*. In the EU, reused water for agricultural purposes should follow specific quality recommendations. For instance, a fecal coliform count of <10 CFU per 100 mL in reused water can be used for food crops with edible parts that come directly into contact with reused water. For edible parts of food crops that are positioned above ground, reuse water should pose a fecal coliform count of <100 CFU per 100 mL (Alcalde-Sanz & Gawlik, 2017).

Conclusion and Future Recommendations

Environmental monitoring in the food industry is critical to ensure food safety and quality. The adoption of biotechnology knowledge has set forth food manufacturers to effectively detect potential hazards, verify cleanliness and sanitation, and remove pollutants in wastewater. Nevertheless, continuous improvement and intervention must be implemented soon to face ongoing socio-economic challenges and fast-changing adverse climate effects to uphold food safety and security.

The food industry has evolved through a series of revolutions, from mechanization, electrification, automation, and now digitalization. Digitalization is embedded

in major industrial change, known as Industry Revolution 4.0 (IR 4.0). IR 4.0 emphasizes using robotics, 3D printing, mobile technology, and sensors to boost industrial production (Dadhaneeya et al., 2023). IR 4.0 has provided a good platform for developing rapid/alternative techniques to detect microbial contaminants and allergen residues with sufficient precision and promptly. Biosensors are seen as a promising method that is aligned with IR 4.0 movement. Future researchers may aim to invent reusable detection kits for sustainability and reduce cost and energy efficiency while maintaining the sensitivity, selectivity, and precision of the test.

A risk-based approach is widely applicable as the food industry is pursuing prevention rather than a responding paradigm. Zero risk may not be attainable in an ideal world, but the use of predictive modeling can be used as a powerful tool to assist food manufacturers in estimating risk. Tracing patterns of microbial contaminants in food processing niches could be explored using Artificial Neural Network (ANN). Microbial contaminant detection using Artificial Intelligence (AI) can offer more control by food manufacturers to monitor the safety of the food environment, thus identifying the source of contamination as soon as possible.

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Chapter 8
Biofilms on Food Contact Surfaces: Current Interventions and Emerging Technologies

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Introduction

Microorganisms are omnipresent. Owing to this ubiquitous nature, microorganisms are one of the most significant bumps for food processors. The prominent threats to the quality and safety of food products are the unfavourable microbial contamination of food and food contact surfaces. Apart from these two, today's food processors must overcome numerous other obstacles to ensure a steady supply of safe and healthy food.

Food contact surfaces are either "open" or "closed" type in the food industry. Pipework is a typical example of a closed surface because it contains ingredients or wet products in a flowing liquid system. Open surfaces are exposed and moist or dry food moves down conveyors, flow is absent because the liquid is not required to surround the food or cover the surface (Verran et al., 2008).

Closed systems avail solid-liquid interface for attachment and colonization of microorganisms. Such circumstances readily promote the development of biofilms in closed systems. Solid-air or a solid-liquid-air interface of open systems is comparatively less vulnerable to microbial attack as they may undergo dehydration, lack of moisture, and be subjected to routine cleaning and sanitization process. Because nutrients are concentrated more near an interface, it is metabolically advantageous for bacteria to adhere to surfaces. Once anchored, bacteria will consume and metabolize nutrients, excrete waste, and build structures or substances that promote

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adhesion and reproduction. A dynamic three-dimensional network is created as a result. The conventional and newly developed methods used currently in the removal of biofilms on food contact surfaces are discussed in the chapter. We explore both the thermal and nonthermal approaches for reducing the microbial load, highlighting the existing technologies and their drawbacks. The chapters also cover emerging technologies ranging from antimicrobial coatings to cold plasma technology.

Biofilms

Flemming and Wingender (2010) have defined biofilms as a group of microorganisms adhering to surfaces, or to one another and covered by extracellular polymeric substances (EPS). A biofilm is a stable community comprising both biotic and abiotic components and its formation in food processing environments has significant adverse effects. Hence, regular cleaning and disinfecting processes are necessary for safely operating open systems. Biofilms are formed in moist and non-sterile environments. One of the most common example is the dental plaque which leads to tooth decay and gum disease. These are caused by the metabolic by-products of the bacteria found in plaque. The human skin lining also serves as an excellent reservoir for biofilms.

Microorganisms, in the form of biofilm or otherwise, can multiply and colonize when they are transferred from an inert surface to a food milieu, which increases the risk of contamination, spoilage, and/or disruption of quality assurance operations. Development and retention of biofilms in the food industry are influenced by a variety of elements such as the topography, chemistry and configuration of the surface as well as the type, physiology, and viability of microbes, and interactions between these elements. These must be taken into account while deciding about the techniques to be used to determine the effectiveness of sanitation.

Occurrence of Biofilms and Associated Risks

The majority of the biofilm is made up of extracellular polymeric substance (EPS), which is typically made up of polysaccharides, proteins, glycolipids, enzymes, metal ions, and nucleic acids in the biological environment. Over 80% of the biofilm's volume is made up of EPS, and this component's physical and chemical makeup influences the biofilm's basic traits and features (Branda et al., 2005).

Food Industry Biofilms may form on surfaces in the food business that come into contact with or are not in contact with foods. About 60% of foodborne outbreaks are caused by biofilms (Han et al., 2017). The biofilm formation in areas where food is processed puts consumers' safety and the food business at serious risk. Contaminants in the surroundings where food is processed typically come from the air, equipment,

or food surfaces. These contaminants lead to biofilm formation in food processing environments and may cause food spoilage, pose a major risk to the health of consumers, and cause a negative economic impact (Coughlan et al., 2016).

Foodborne illnesses caused by microbes are a big problem, including *Salmonella* spp., (causes Reiter's syndrome or even death); *Escherichia coli* 0157:H7 (causes hemorrhagic colitis) (Wirtanen & Salo, 2016); *Listeria monocytogenes*, (causes abortion in pregnant women and other difficulties in immunocompromised patients). This foodborne pathogen is most commonly associated with biofilm formation and also food spoilage (Galié et al., 2018); *Vibrio parahaemolyticus*, (associated with seafood infection on the consumption of undercooked seafood; *Clostridium perfringens* (a toxin-producing species); *Campylobacter jejuni* (causes human gastroenteritis); *Pseudomonas* spp., (produces proteases that leads to food spoilage); *Bacillus* spp. (responsible for diarrhea and emetic sickness); *Staphylococcus aureus* (responsible for foodborne intoxications); *Shewanella putrefaciens* (produces putrid odour due to production of volatile amine, sulphide, and trimethylamine) (Bagge et al., 2001); *Geobacillus stearothermophilus* (a typical dairy product contamination); and *Cronobacter* spp. (a species mostly responsible for infant infections and immunocompromised individuals). These organisms can even create multi-species biofilms, another significant technical issue facing the food sector, which is more persistent and challenging to regulate (Galié et al., 2018). The presence of biofilms hinders the transmission of heat through equipment, corrodes the surfaces, and also increases the resistance to fluid friction at the surfaces and thus decreases production efficiency.

Medical Facility The formation of biofilms affects human health directly as well as indirectly. It is directly associated with chronic illnesses in humans such as cystic fibrosis, prostatitis, dental caries, rhinosinusitis, and otitis media. Additionally, these films cause a lot of indwelling medical devices to malfunction and raise the risk of bloodstream infections through catheters (Percival, 2007). Over 65% of infections by microorganisms are thought to be caused by the ones associated with biofilms, and these species have significant resistance to antimicrobials and elements of the host defense system (Jamal et al., 2018).

Others Human diseases and waterborne outbreaks have been linked to ingesting water polluted with bacterial biofilms. The major biofilm-producing bacteria in the drinking water system are *Pseudomonas aeruginosa, Aeromonas hydrophila, Klebsiella pneumoniae, Campylobacter jejuni, Legionella pneumophila,* and *Mycobacteria* (Chan et al., 2019). Bacterial cells may cause corrosion of water pipes, and affect water quality in terms of color, taste, turbidity, and odors, as well as its efficiency of heat exchange. This may happen when these organisms attach and form biofilms on the interior surfaces of pipes, from which cells break and enter the water supply (Prest et al., 2016). Overall, biofilms can harm water pipes and have a negative impact on the safety of drinking water.

Microbial Attachment and Development of Biofilm

Food and its contact surfaces can get contaminated with both pathogens as well as spoilage-causing microorganisms which enter through soil, water, tools, people, animals and air. For the food sector, the adhesion of microorganisms, particularly bacteria, and subsequent biomaterial accumulation has been a major source of issues. The type of microorganism responsible for developing a biofilm depends on the type of food substrate. For example, in the closed systems found in the dairy industry, thermophilic streptococci are related to product spoilage and pose a threat to the cleanability of surfaces (Flint et al., 2000). *Listeria monocytogenes* being a psychrophile pathogen is of concern in the cheese, meat and fish industries (Tresse et al., 2007). Studies have shown that biofilms containing *Listeria monocytogenes* could form on surfaces such as walls, floors, and in drains of food processing plants. A biofilm may be constituted either by only one microbial species or by a combination of a number of species belonging to different microbial groups including bacteria, yeast, fungi, algae, and protozoa. These microbes attach firmly to and have complex interactions among themselves and with the surrounding abiotic environment (Costa-Orlandi et al., 2017; Raghupathi et al., 2017). Biofilms can be formed on all food processing surfaces and equipment.

The ability of bacteria to form a particular type of biofilm will influence their persistence during manufacturing and retail. It will also influence the rate at which they cause infection. The movement of food through the processing lines or its handling in the processing, environment may cause the surface conditioning at a solid-air contact to be more generic rather than specific in nature. Food-substratum contact is another opportunity for microbial contamination and biofilm formation.

Apart from carrying pathogenic microbes, the formation of biofilm increases the frictional resistance, and thus, the cost of the process. Biofouling of heat exchangers can result in a reduction in heat transfer efficiency and an associated cost increase. Thickened biofilm may also corrode pipeline surfaces. An area beneath a microcolony becomes anodic in comparison to the rest of the metal, which is exposed to the oxygenated bulk phase. And then the associated bacteria in the biofilm deplete the oxygen near a metal surface, thus causing corrosion.

The contact surface must be inert in order to prevent the transfer of any potential contaminants to food. Stainless steel is a material of choice in the food sector as it is non-reactive, non-corrosive, and stable. It can undergo various processes such as electrolysis and mechanical transformations to form a variety of surfaces and products (Verran et al., 2004). The likelihood of contaminating germs penetrating a material increases when its flexibility is lost due to excessive wear and the ensuing breaking, which is most commonly seen in surfaces or equipment that use rubbers, plastics, epoxy resins, etc. Even cracks and slits in stainless steel and glass can serve as a reservoir for pathogens.

Additionally, temperature, chemistry, and processing of food determine the strength of attachment. All these factors drive the type of method employed to disinfect the food contact surfaces.

Mechanism of Microbial Attachment

The complicated biofilm formation process commences with the attachment of a single bacterium. The duration of this process may vary from very short to long in the food processing environment depending on the surrounding medium, the surface involved, and the type of associated microbe. The process of biofilm formation is complex, and occurs in several steps starting with the attachment of microbes reversibly to surfaces through intermolecular forces and hydrophobicity followed by the release of extracellular polymeric substances (EPS) enabling permanent adherence of the cells to a surface (Caruso et al., 2018). The process of biofilm formation usually involves five main phases that include: (i) Reversible attachment, (ii) Irreversible attachment, (iii) EPS production, (iv) Maturation of biofilm, and (v) Dispersal/detachment (Stoodley et al., 2002; Toyofuku et al., 2016).

The microbial cells, at a distance of more than 50 nm, are attracted toward the surface via hydrophobic interactions and van der Waals forces. The electrostatic force comes into play when the bacterium advances near (<20 nm) to the substratum and is the strongest force responsible for the adhesion of microbial cells. As the cells approach even closer, it marks the irreversible attachment. Studies have shown that the rate of attachment of spores is greater than vegetative cells, because of their higher hydrophobic nature and non-uniform outer surface. After the subsequent attachment, the microbial cells multiply and propagate, leading to the growth of colonies, and thus a biofilm is formed.

Factors Affecting Retention

A standard 8–12 h shift in the food sector gives bacteria plenty of time to adhere and build a biofilm on process equipment in just a few hours. Some surface characteristics are important to support bacterial colonization. These include the hydrophobic nature of the surface, its topography especially if its non-uniform, and its predisposition for protein adsorption. However, a variation has been found in the behaviour of different surfaces toward biofilm formation. It has been found that rough surfaces retain bacteria better than smooth ones while some studies have shown that there is no effect of rough surfaces (Flint et al., 2000; Medilanski et al., 2002). The retention of biofilm on the surface depends on several topographical and chemical factors.

Substratum Topography

The adherence of microorganisms on the surface increases directly in relation to the surface roughness of the surface. This can be attributed to the fact that a rougher surface has a greater surface area and low shear force. The rate and degree of

adhesion may be significantly influenced by the surface's physicochemical characteristics. Most studies indicate that adherence of microorganisms is faster to nonpolar, hydrophobic surfaces(e.g., Teflon) as compared to hydrophilic surfaces(e.g., glass or metals). Glass and other hydrophilic materials are widely utilized for surfaces that will come into touch with food because they are known to prevent bacterial adhesion. Hydrophobic spores were discovered to stick to both surfaces more readily than vegetative cells did. Passive retention will be minimal if the surface irregularities are bigger than the microorganisms. The retention hence improves if the surface features are of a similar dimension or slightly smaller.

The variability of surface micro and macro-topography adds another element to be paid attention to while making surface microbes free. The rinsing process might prove to be more effective when done along with the direction, that is, parallel to the cracks or crevices rather than in a perpendicular direction.

Also, different microorganisms portray different attachment behavior toward different surfaces. *Pseudomonas spp.* gets attached to glass surfaces in the dairy industry more readily than others. The biofilms found in milk processing systems harbored *Acinetobacter spp.* even though it has a predominately gram-positive microflora.

Surface Chemistry

Due to the various chemical and physical properties of food ingredients, the contact surfaces must be non-reactive to avoid any distortion, via chemical reactions, in the final product. In the food industry, the transfer of any chemical substance from the food surface is undesirable. Various studies have shown that the chemical properties of contact surfaces and microorganisms have offered us ways, like coating surfaces with specialized substances at critical points, to ensure food safety.

Stainless steel is the most chosen metal for use as a contact surface in the food sector as it is noncorrosive, non-reactive and long lasting. Its chemical composition also allows a wider functionality, for instance, chromium increases corrosion resistance (Maller, 2007). Chromium undergoes passivation when it interacts with the atmosphere. The layer of oxide, thus formed, gives noncorrosive properties to stainless steel (Olsson & Landolt, 2003). Nickel, manganese, and molybdenum, for instance, can be included. There hasn't been much research done on how variations in stainless steel's surface chemistry affect microbial retention.

The role of surface chemistry in microbial attachment has been studied by many researchers using various surfaces having similar topography. Glass surfaces having different chemical groups with varying hydrophilicity, hydrophobicity, chain length, and chemical functionality were used to study the adhesion of *Listeria. monocytogenes, Salmonella typhimurium, Staphylococcus aureus*, and *Escherichia coli*. It was seen that the chemistry of the underlying substratum affects the adherence of *Listeria spp.* and *E. coli.* Teughels et al. (2006) have shown that the formation of biofilm is also affected by underlying surface chemistry.

Availability of Organic Matter

The habitation of organic and inorganic matter on contact surfaces affects both, the formation of and its cleanability. The presence of soil hinders the action of cleaning agents physically and chemically, thus giving favorable conditions for microorganisms to flourish. The organic material might act as a nutrient medium for microorganisms to grow, thus contaminating food. The biological surface that the conditioning layer provides to microorganisms at the solid–liquid interface by means of receptors is responsible for providing a degree of specificity (Verran et al., 2001). This may occur through direct food contact in an open system (the solid-air interface), which is more common in the sector. Surface features may contain attached microorganisms mixed with organic materials like fats, carbohydrates, proteins, or detergent residue. Surface conditions, and consequently microbial attachment and retention, get affected by cumulative soiling. Verran et al. (2002) have stated that "soiling" may be used where the surface is uneven and significant exchange of organic matter occurs rather than merely "conditioning of film".

Assessment of Retention

It is generally known that bacterial cells that form a biofilm matrix and colonize surfaces possess greater resistance to toxic chemicals compared to their single-cell existence. In a biofilm, the bacterial cells receive less oxygen and nutrients. This leads them to undergo some major physiological changes, which results in their decreased growth rate. This quasi-dormant nature of bacteria in a biofilm is responsible for their resistance to a variety of antibiotics, surfactants, and sanitizers.

It may be feasible to hypothesize about the biocide resistance demonstrated by microbes attached to surfaces by examining microbial cells in terms of their structure and functions. Some species, like *Bacillus* spp., have a proteinaceous capsule that aids in adhesion, stops desiccation that blocks the action of phagocytes. Most bacteria's extracellular capsules are polysaccharides by nature. Capsular material may also make it easier for harmful substances to be absorbed, restricting their entry into the cytoplasm. Hence, a biofilm consisting of capsular material would offer protection to embedded cells against sanitizers.

Research has shown that the resistance of bacteria to disinfection depends upon their surface attachment. For example, a disinfecting agent can attack planktonic microorganisms from all sides as they are free floating. The microorganism which is attached to a surface is affected only from one side but removal from the surface increases its susceptibility to sanitizers.

Age of biofilm often improves the bacteria's resistance when present in biofilm. The older the biofilm is, the greater is the resistance to various sanitizing agents. A biofilm develops multiple layers as it ages as a result of imprisoned cells' growth and reproduction. It has been discovered that sanitizers, such as quaternary

ammonium compounds (QAC), are useless against biofilm cells that are present beneath the initial layer. QAC should permeate hydrophilic and negatively charged cell surfaces since they are hydrophilic cationic molecules. However, lipophilic surfaces as in the cell wall of Gram-positive may prevent sanitizers from penetrating.

Cells in a biofilm may develop sanitizer resistance through a surface-dependent mechanism. Researchers have discovered that the type of surface to which *L. monocytogenes* had adhered was related to the bacteria's resistance, and they came to the conclusion that in comparison to surfaces made of polyester or polyester-polyurethane, stainless steel surfaces were much easier to clean and sterilize. No discernible topological changes among the surfaces were found by scanning electron microscopy to explain this variance in sanitizing resistance.

The way the bacteria in biofilms resist antibiotics appears to be comparable to how they fight sanitizers. *Staphylococcus aureus* cells from old biofilms were shown to be extremely resistant to tobramycin and cephalexin. The process underlying this resistance has been hypothesized to involve modifications of the antibiotic's ability to pass the cell membrane, the development of enzymes that break down antibiotics, alterations to the molecular targets of the antibiotics and prevent penetration by binding of bacterial exopolysaccharides to the antibiotics. Similar behavior of antibiotics like amikacin were observed when their actions were studied on the suspended and adhered *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*.

Decontamination of Food Contact Surfaces

Any surface to which food substances contact during preparation, manufacturing, processing, and packing are considered as food contact surfaces. Usually, stainless steel or some other variety of plastic is used for these surfaces, but other materials, such as wood, rubber, ceramic, or glass, may also be used as contact surfaces. All these surfaces could be a great source of microbial contamination as already discussed in this chapter. Recent evolution in the food industry has paid significant attention to the methods used for decontaminating these contact surfaces.

The term decontamination refers to microbial inactivation or removal by a process of disinfection or sterilization. The reduction in the number of microorganisms usually by destructing or removing the vegetative forms of bacteria, fungi, and other microbes from inanimate objects by using a number of chemicals alone or in combination is called disinfection; the spores generally remain on using disinfectants. On the other hand, in sterilization there is complete destruction of all the microorganisms, not only the vegetative forms but their spores as well (Skåra & Rosnes, 2016). These microorganisms if not removed, particularly the pathogenic ones, develop biofilms, which are composed of single or multiple species, over and/or around these food contact surfaces. Studies have proven that these biofilm pathogens have developed resistance against many of the existing antimicrobial agents. The arising antibacterial surface designs provide the chance to lessen or eliminate microbial adherence (Sharma et al., 2022).

Types of Methods

There are numerous ways to decontaminate a food contact surface, and the ones often used employ different chemicals like chlorine and quaternary ammonium compounds. Use of chemicals directly on food-contact surfaces needs approval from FDA. The industry usually prefers to use different chemicals together or in a sequence and use multiple methods to reach /get the required level of decontamination. The most important principle when it comes to surface decontamination is the prevention of recontamination.

Broadly, decontamination methods can be divided into two classes based on the ways the exposure to heat happened. These classes are designated as "Non-Thermal'' and "Thermal" methods. It can be easily witnessed that a combination of both the aforementioned methods are used in the industry in their routine surface sanitation, both in CIP (Cleaning in plant), as well as in COP (Cleaning out plant). Each of these classes encompass several methods which are discussed here further.

Non-thermal Methods

- *Chemical Methods*

Chemical methods of decontamination require the use of a chemical agent or a mixture of multiple agents. They are popular because of their highly effective fungicidal, bactericidal, sporicidal, and antiviral effects, out of which the latter are considered among an exclusive class of disinfectants (Skåra & Rosnes, 2016). These are one such methods which are practiced by almost everyone in the business of food and related domains. But not any chemical agent can be used to meet the purpose, only the approved chemical agents which are allowed as per the regulations, which vary from country to country, can be used for food-related surfaces. When they are used excessively, there are also high chances of the residues of these chemicals being left behind and this raises a concern about whether one should continue to use these agents or not. But this can be tackled easily if the personnel in charge of the sanitation duty ensures that only the approved chemical agents are used for the decontamination, with all the instructions being followed, from concentration to rinsing and drying. The efficacy of these chemicals depends on factors like concentration, contact time, temperature, surface area and its nature, organic and inorganic content etc.

Some common agents used to decontaminate food contact surfaces include Chlorine compounds (e.g., Calcium and sodium hypochlorites), Peroxide and Peroxyacid mixtures (PAA), Quaternary Ammonium Compounds (QUATs), Iodophors, Hydrogen peroxides, and others. Many newer methods have come up to replace these agents in recent times. Some of these new technologies are described in the following sections.

- *Physical Methods*

Physical methods of decontamination are commonly used with the thermal methods, and many times considered the same. Though physical methods are not very effective against disinfection except for food removal of soiling and some microorganisms from the surfaces, they are involved in assisting other methods of decontamination. This is because physical methods are applied prior to any other methods and thus reduces some microbial load and the number of interfering agents which could hinder the efficiency of the other methods which will be used next in the sequence. The use of both ionizing (e.g. gamma rays) and nonionizing radiations (e.g., ultraviolet rays) is also considered to be a physical method of decontamination.

Thermal Methods

Thermal methods include the application of heat energy. It is an inefficient method of decontamination as it uses up a lot of energy in its operation. Number of factors including temperature, relative humidity, duration of exposure/exposure time, etc. influences the efficacy of heat in destroying the microorganisms. It is found that the combinations of heat and other decontaminating agents are preferred as rise in temperature enhances the rate of reaction and thus efficiency of many agents. The most common agents used to provide heat energy are air, steam, and hot water, out of which, the use of steam is more common. Steam is considered to be an efficient carrier of heat energy when applied on the food contact surface, but limitation lies in difficulty in monitoring the exact contact time of steam with the surface and the temperature used and also using steam to meet this purpose is expensive.

Immersing small equipment in hot water, which is heated at or above 82 °C, is a good way of sterilizing cleaned components. The exposure time needed to remove microbes completely from any item is dependent on the water temperature used. Lower the temperature of steam, longer would be the time required to sterilize the equipment or contact surfaces. The time required would be shortened at a higher temperature.

Existing Technologies and Their Drawbacks

Since ages, many techniques have been practiced tackling the problems related to Food Contact Surfaces. Existing methods generally prove to be of little help, though some may have greater impact, but no method is perfect. CIP (Cleaning-In-Place), a very common approach to deactivate bacteria on food contact surfaces, which is still being applied by most of the food companies and uses a number of rinse cycles repeatedly, has failed to provide a clean and safe surface against planktonic cells of the bacteria.

In the food industry, applications of radiation, heat, and chemicals are looked for in disinfection and sanitation. The former (radiations) being expensive, heat and chemical methods are more prominently used. Chemical compounds like Iodophors and Chlorine compounds, often fail to fulfill their purpose when they interact with the food residues and dirt already present on these surfaces. Their efficiency is decreased, and they fail to disinfect the surface properly (Sharma et al., 2022). Chemical fogging is a method of decontamination that has been found to be helpful in the disinfection of water, equipment, and food surfaces (Beltrán et al., 2005; Gelman et al., 2005). It is of great interest to the food scientist and has been in focus because of its low toxicity. It is used for decontaminating whole rooms and has been very effective (Nicholas et al., 2013; Zoutman et al., 2011). But its high effectiveness comes with a cause of its high toxicity which raises a question on its use in areas inhabited by people. Because of this reason, their use is restricted to areas that can be separated during decontamination and thoroughly vented afterward, or where it is possible to give sufficient time to the gas to degrade.

Emerging Technologies

Keeping in mind the demerits associated with conventional decontamination methods, a number of advanced technologies using ultrasonication, cold plasma, and surface functionalization are emerging that hinder microbe's attachment to FCS and promote microbial killing, thus reducing contaminants and enhancing food safety and its quality (Fig. 8.1). Some of these include:

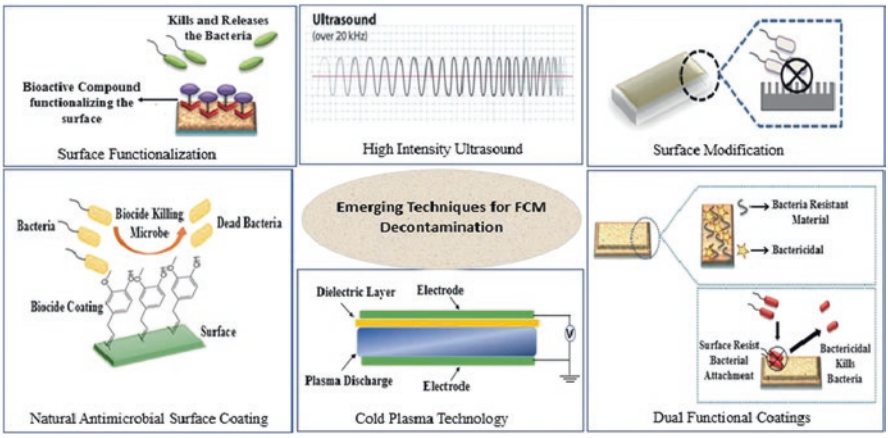

Fig. 8.1 Emerging techniques for FCM decontamination. (Source: Sharma et al., 2022)

Natural Antimicrobial Surface Coating

The term "antimicrobial coating" refers to a liquid combination, solution, or suspension that is administered to a surface with the intention of sanitizing, disinfecting, decreasing, or moderating microbial development on surfaces that come into contact with food.

Antimicrobial surface coatings are based on achieving either or both of the following motives. One that prevents the primary attack by microbes and their spores, by generating surfaces that do not permit the adherence of the microbial cells and their spores. The second one is based on the killing of approaching microbes (Tiller, 2010). The properties of an ideal antimicrobial coating as per Provider and Baghdachi (2008) are:

- It should be effective.
- It should be both mechanically as well as chemically resistant.
- The coating should be stable, non-absorbent and innocuous.
- It should be cost effective.
- Easy to clean when placed in a complex environment.

There are five guiding principles that cover all the interactions with microorganisms that result in the suppression of microbial development on food contact surfaces and may be used to categorize the possible uses of antimicrobial coatings. These include

- Anti-adhesion: Modification of surface energy to develop passive repellence.
- Antimicrobial-loaded:
  - Simple release: There is continuous release of the agent present in the matrix until depletion.
  - Controlled release: The antimicrobial located in matrix is released against a stimulus until fully consumed.
- Contact inactivation: Lysis of cell occurs when it comes in contact with active agent.
- Photocatalytic: The damage to the cell wall occurs due to the reactivity of various oxygen species which are released continuously.
- Multifunctional: Different types of action mechanisms are working together to reduce the microbial load (Torres Dominguez et al., 2019).

Animals, plants, bacteria, fungi, and algae are just a few examples of the many sources from which antimicrobial agents can be derived. Plants are found to have polyphenols as their secondary metabolites. These polyphenols are generally subcategorized as flavonoids and non-flavonoids. There are numerous studies that support the efficiency of these polyphenols and essential oils when incorporated in antimicrobial coatings in food contact surfaces. Vazquez-Armenta et al. (2018) studied the effect of extracts obtained from the grape stem on *Listeria monocytogenes* attached to stainless steel and polypropylene surfaces. They studied the factors like motility, surface energy and adhesion. The essential oil from *Origanum*

vulgare L. (oregano; OVEO) and carvacrol (CAR) was used for the removal of biofilms formed on stainless steel surfaces by Dos Santos Rodrigues et al. (2018).

Dual Functional Coatings

In recent times, a number of antibacterial surfaces have been formulated. These surfaces or coatings have been classified on the basis of their mode of action by Yu et al. (2015). They are classified are:

(i) Surfaces which are bactericidal in nature
(ii) Surfaces resistant to bacteria
(iii) Surfaces which do not permit the attachment of bacteria

Surfaces with dual functional coatings combine both bactericidal and microorganism-resistant qualities. Ahmadi and Ahmad (2019) created a polyurethane nanocomposite (PUC) covering that is durable, active, and dual-functional (antimicrobial/anticorrosive) by combining the synergistic activity of graphene oxide incorporation and π-π interaction. This covering reduced the bacterial colonization against *Salmonella typhimurium* and *Listeria innocua* as compared to planar aluminium and also had anticorrosive action. A large number of studies have been done to develop a surface coating having dual functionality.

Surface Functionalization

Research in the area of surface modification has led to many techniques which have improved both the inertness and safety of the materials commonly used in making of food contact surfaces. Different agents such as UV rays, wet chemicals, and adhesion techniques can be used to add various polar groups to the surface and create different functional surfaces. Different factors such nature of material, and its properities including conductivity, strength etc., have to be considered while selecting the polymer. This is the initial stage in the surface modification process which is followed by the addition of appropriate quantity and type of the reactive functional group. This second stage helps in increasing the surface functionality. The use of the surface decides which type of biomolecules to be immobilized or its functionalization. The availability of the reactive functional groups per unit area is increased by grafting the polyfunctional agent onto the surface. The bioactivity is also increased by adding spacer molecules to utilize the bioactive compounds on a solid surface. Hence, the steric hindrance is decreased and the compound is coated with a hydrophobic substance. The last step is the covalent bonding of the natural or synthetic bioactive compound to the functionalized polymer surface.

Atom radical transfer or covalent bonding are the two techniques of achieving surface modification. In a study, antibacterial property was achieved by using hydrophobic polycations of quaternary ammonium salt which were chemically bound on surfaces (Sharma et al., 2022). Perinelli et al. (2019) showed that the specific properties such as cytotoxicity, and antibacterial activity of surfactants having quaternary ammonium amino acids are affected by the length of the hydrocarbon chain. On the other hand, they are unaltered by the polar head of the amino acid-leucine or methionine.

Surface Modification

Biofilms contaminate food and its product because the bacterial population has a tendency to adhere and colonize material surfaces, thus forming a biofilm. Few studies have been conducted to determine the effect of surface topography of the material on the bacterial adhesion. Hsiao et al. (2014) showed that the surfaces which are smooth and convex are more resistant to the bacterial cell attachment. The indented surfaces have better anti-biofouling properties as compared to the curved surfaces as the bacterial cells cannot adhere in between the indents (Hasan & Chatterjee, 2015). The role of food-safe oil-based anti-friction coatings (FOSCs) was studied by Awad et al. (2018) in preventing biofilm formation, this was attributed to the fact that the residual oil formed a coating on the surface holes thereby stopping the microbial growth as no anchorage was available. Their findings indicated that the film formation on stainless-steel food contact surfaces can be reduced by employing cheap yet sustainable approaches which in turn do not allow the biofilm formation and enhance the safety of food.

High-Intensity Ultrasound

In recent years, the food sector has paid a lot of attention to the use of ultrasound. Ultrasound is considered a green and cost-effective technology (Zheng et al., 2019). The decontamination of surfaces submerged in an ultrasonically activated liquid is easily achieved by the application of high-intensity, high-frequency sound waves. The ultrasound has become more popular as a technique due to better understanding of chemical reactions and surface conditioning in recent times. This technique is non-destructive and offers many benefits such as uniform cleaning, microbiological safety, and maintains quality of food (Sharma et al., 2022).

The ultrasonic waves are made up of compression and expansion cycles. They have the ability to penetrate and disinfect any surface which is submerged in a liquid medium that conducts sound. These waves can reach difficult areas and expertly clean tread roots, blind holes, and even the smallest surface shapes. Yu et al. (2020) stated that positive pressure pushes all the molecules together during compression

whereas expansion cycle creates voids due to the created negative pressure which overcomes the tensile strength of the fluids. This makes ultrasonic cleaning a very effective and distinctive method.

Reduction in the attachment of biofilms is one of the main features of ultrasonic cleaning. Brasil et al. (2017) evaluated a non-thermal method of cleaning and disinfecting knives used in slaughterhouses in combination with ultrasound using chlorinated water and a neutral detergent. The temperature required to clean and decontaminate cutlery was reduced after the ultrasonic treatment. The cleaning process of the knives was more effective and ultrasound had no effect on the structural integrity of cutlery tested.

Cold Plasma Technology

Plasma-based technology is a unique and non-thermal method used for the production of antimicrobial materials and can be used as an antimicrobial coating. These coatings can be used on different types of metallic, polymeric and ceramic surfaces also. These can be applied even on temperature and moisture reliant devices. This is not doable using wet technology. Niemira and Gutsol (2011) have stated that terms like cold, cool, and non-thermal plasmas are applicable to the procedures operating at or near room temperature whereas thermal plasmas are associated with arc welders, combustion tools, or other high-temperature methods. Cold plasma is a non-contact, waterless technology and does not employ any antiseptic substances but an effective decontamination technique. Therefore, food contact surfaces that are prone to contamination with human pathogens can be treated with cold plasma. Romani et al. (2020) found that the treatment with plasma and carnauba wax coating on bi-layered myofibrillar protein film improved its tensile strength and water vapor permeability for its use as packaging material.

The current focus is on the plasma polymer film, which is made by plasma-enhanced chemical vapor deposition. These films exhibit exceptional adhesion, a highly crosslinked structure, and the capacity to change attributes by varying a parameter or precursor. Additionally, they are suitable for industrial use, due to the reduction in the use of chemical solvents. Advanced composite films or complex hybrids can be synthesized when plasma source parameters and deposition structure are properly controlled. Due to their abundance of free radicals, polymers that have been plasma-treated or created serve as highly reactive surfaces.

Prevention

When a food contact surface is decontaminated, the following core task is to prevent any recontamination from occurring, either on the same surface or on the finished product. This can be achieved by the complete removal of the source or potential

source of recontamination from the whole processing line. Altering the environment in which the processing is done can also help in the prevention of recontamination. Microbes generally grow in places of high moisture so removal of moisture becomes a key point in preventing recontamination. All the places, where there are chances of water being found stagnant, must be identified and must be taken care of. Similarly, some dead ends are not properly cleaned or are left unintentionally, as a result, these places start harboring microbes that can eventually cause failure in the decontamination systems. Changing the materials of the equipment could be really helpful as well, for example, replacing conveyor belts with stainless steel would prevent microorganisms from attaching to the surface, hence lowering the chances of contamination during the process.

Conclusion

The microbial contamination in the form of biofilm formation on the food contact surfaces is detrimental to the efficacy of the contact surface in terms of both high or low temperature exchange as well as poses health hazards to the end consumers. Food scientists and technologists have worked for years on developing methods that may reduce the microbial attachment or create an antimicrobial environment using layers which are both functional as well as safe. The research has resulted in generating a number of solutions and technologies to resolve the problem of biofilms. Cold Plasma technology, High Intensity Ultrasound, and Surface functionalization are some technologies that are revolutionizing nature and usher in the change in tackling this issue. Their efficiency can be increased further by incorporating two or three technologies together to generate better results. Multifunctionality is the need of the hour. The surface coating should be multifunctional yet safe for human health. Therefore, incorporating natural compounds into the surface's growth is and would be the best way to enhance the sanitization process and change the physicochemical characteristics of the surface. However, more research is needed in the field of safety and inertness of the food contact material. The quality of the food must be retained along with increasing the safety and inertness of the food contact material. There is an urgent need for a multifunctional antimicrobial coating having a nanoscale surface topology may find use in all aspects of food processing. Additionally, combining two treatments, such as using steam followed by ultrasound, could be a practical choice. A significant focus should be placed on using more natural agents or their extracts for decontamination purposes as consumers are looking for natural or organic solutions. There is an increased awareness and consumers nowadays are familiar with the toxic effects as well as carcinogenicity of some detergents and cleaning materials which might find entry into the food indirectly and make food unsafe for consumption.

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Chapter 9
Microbial Biofilms and the Role of Biotechnology as a Solution

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Food Biofilms

What Is a Biofilm?

Many microorganisms have a proclivity to attach to surfaces and form a complex made up of extracellular matrix, in various environmental settings. These complex ecosystems with embedded bacteria, are known as biofilms. Furthermore, microbes in biofilm state often times are quite dissimilar in their phenotypic traits and exhibit high antimicrobial tolerability, In contrast to planktonic organisms (Abdallah et al., 2014; Galié et al., 2018) It has been demonstrated that mixed biofilms are resistant to biocides and disinfectants, such as quaternary ammonium compounds.

Impact of Biofilms in the Food Sector

In the food industry setup, biofilms can prove to be a notorious issue to tackle. The type of surface material and the reversible attachment of microbial cells to it are the initial processes involved. After that, the attachment becomes irreversible, with embedded microcolonies. Finally, the biofilm presents as a tridimensional assembly (Fig. 9.1), creating a sophisticated ecosystem that is prepared for dispersal.

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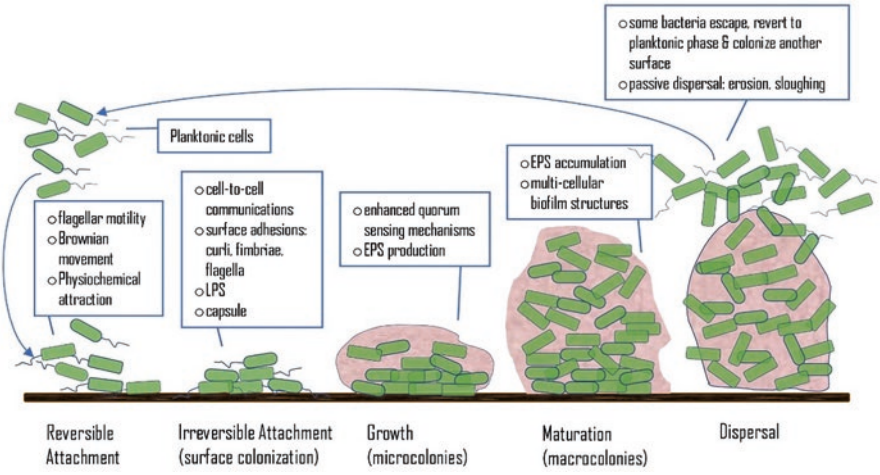

Fig. 9.1 Stages in biofilm development

Attachment Mechanisms

A variety of biological, chemical, as well as physical mechanisms control the formation of biofilms. The terms adhesion and cohesion are used to describe different types of cell attachment in a biofilm. The adhesive and cohesive qualities that a biofilm will display are ultimately determined by the mechanisms underlying these types of attachment. The different steps involved in biofilm formation are (Marshall, 1986):

(i) adsorption, or the buildup of an organism on a substrate or other collecting surface (deposition); (ii) attachment, which is the stabilization of bacterium-collector contact and frequently entails the building of polymeric bridging between the two; (iii) colonization, i.e., the growth and division of microbes on the collector surfaces.

(a) *Conditioning layers:* The conditioning layer, which may be made up of several inorganic or organic particulates, serves as the substrate onto which a biofilm can develop (Characklis & Cooksey, 1983). Through gravitational force or flow movement, anything that might be present in the main fluid may settle down on a substrate and eventually becoming a component of the conditioning layer. This layer changes the substrata to make it readily accessible to microorganisms. Surface charge, potential, and tension can be positively affected by the interaction between the conditioning layer and substrate. The substrate gives the bacterial community support and nutrients to help it grow.

(b) *Reversible adhesion:* Initially, physical traits, including flagella and other bacterial appendages are used to move planktonic microbial cells from the main medium/fluid, to the conditioned surface. Reversible adsorption occurs in a portion of the available bacterial cells that reach the surface. Microbial adherence

is locally influenced by characterizing factors, like the amount of energy available, the functionality of the surface, the orientation of the bacteria, and the temperature and pressure levels. The bacteria will separate from the surface if the attracting forces are stronger than the repulsive ones. This is more likely to happen prior to substrate conditioning.

Bacterial desorption has a low activation energy, making it likely to happen and illuminating the bonds' frailty. DVLO (Derjaguin, Verwey, Landau and Overbeek) forces, also known as van der Waals forces, along with electrostatic (double layer) interactions and steric interactions, are involved in defining the extent of individual bacterial adhesions (Rutter & Vincent, 1984). Van der Waals interactions, whether they be associated with attraction, or repulsion, form an overlap amid the substratum and the cell's electrical double layer. These interactions defined by the DVLO theory, describe cell to flat surface net interactions, as a balance between dual influential factors (Hermansson, 1999). Also, these physical interactions are classified as long-distance forces, or physisorption. Thermodynamic interaction has also been discussed in terms of a modified DVLO theory, which further considers hydrophobic/hydrophilic and osmotic interactions (Chang & Chang, 2002).

(c) *Irreversible adhesion:* Some reversibly absorbed cells undergo real-time immobilization and develop irreversible adsorptive bonds. Fimbriae, flagella, pili and other such bacterial appendages have been said to outweigh the electrical double layer's physical repulsive forces (de Weger et al., 1987). They come into contact with the conditioning layer's overall framework, promoting oxidation, hydration and other such chemical reactions to strengthen the bacterial cell-to-surface interaction. According to some research, the hydrophilic-hydrophobic characteristics of involved surfaces have a significant impact on microbial adherence.

(d) *Population growth:* Daughter cells move out and up from their initial attachment sites to cluster up as even more stationary cells continue to undergo binary division (Hall-Stoodley & Stoodley, 2002). These proliferative interactions typically result in the formation of a mushroom-like structure within the growing biofilm. It is thought that the mushroom-shaped structure enables the delivery of nutrients to microorganisms deep within a biofilm.

A rapid population expansion, also known as 'exponential growth phase', proceeds an initial 'lag phase'. This is dependent on the environment's physical and chemical makeup. The substrate's and bulk fluid's nearby nutrients are used up in the process of the rapid growth. Majority of the chemical or physical activities of the initial attachment stage reach a steady halt, and are replaced with other biological communications. Stronger cell bonds are created as a result of the interaction between polysaccharide intercellular adhesion (PIA) polymer excretions and bivalent cations (Dunne Jr., 2002).

The planktonic and sessile state of a bacterial cell differ in terms of gene expression. For instance, because mobility is limited and no longer required in sessile species, the development of surface appendages is hindered. A number of genes that

produce excretion products and proteins on cell surfaces are simultaneously expressed more frequently. Opr C and Opr E are examples of surface proteins (porins) that facilitate the outward passage of excretory products (e.g., some polysaccharides), as well as inward passage of necessary extracellular products into individual bacterial cells of biofilms (Hancock et al., 1990).

Final Stages of Biofilm Formation

When cell division and cell death rates are identical, a phase of growth is said to be stationary. Biofilms employ various cellular signaling pathways known as quorum sensing, which become active at significant cell densities (Bassler, 1999). The process of quorum sensing stimulates genetic expressions of mechanical, as well as enzymatic processors of alginates. These play vital roles within the extracellular matrix and consist of diverse auto-inducers, including chemical and peptide signals such as homoserine lactones, which are present in elevated concentrations.

The biofilm is destroyed during the dying phase. By breaking down the polysaccharidal bindings, the biofilm, together with the help of available enzymes nearby, is capable of causing the surface bacteria to be actively released for colonizing onto new substrates. Enzymes involved in the breakdown of the biofilm matrix include alginate lyase produced by Pseudomonas (Ps.) aeruginosa and Ps. fluorescens, hyaluronidase derived from Streptococcus equi, and N-acetyl-heparosan lyase sourced from Escherichia coli (E. coli) (Sutherland, 1999). Various porin-related genes are down-regulated; they play a role in the genetic cycle for adherence and cohesive properties in different biofilms. On the other hand, flagellin-related operons are up-regulated; they provide the bacteria with the machinery for motion.

Resistance Mechanisms

Increased antimicrobial resistance is a problematic property of biofilm-bacteria. Microbes exhibit strong resistance when attached in form of biofilm, making bacterial cells 10–1000 times more resistant to different antibiotics, as compared to the same bacteria produced in free-floating, planktonic forms (e.g., in culture) (Mah & O'Toole, 2001). Major mechanisms involved in this resistance are briefly shown in Fig. 9.2.

(a) *Difficult antibiotic penetration of biofilm:* The exopolysaccharide matrix (EPM) is a vital feature of any microbial biofilm. A proposition suggests that the EPM serves multiple functions, including acting as a barrier to prevent antibiotics from reaching the bacterial cells. The distribution of antimicrobial compounds, towards the biofilm's cells, can be constrained by either the compound's interaction with or sorption to various biofilm constituents. Although past mathematical models initially predicted that there shouldn't be any obstacles to the diffusion of many antibiotics through a biofilm, modern trials have revealed the

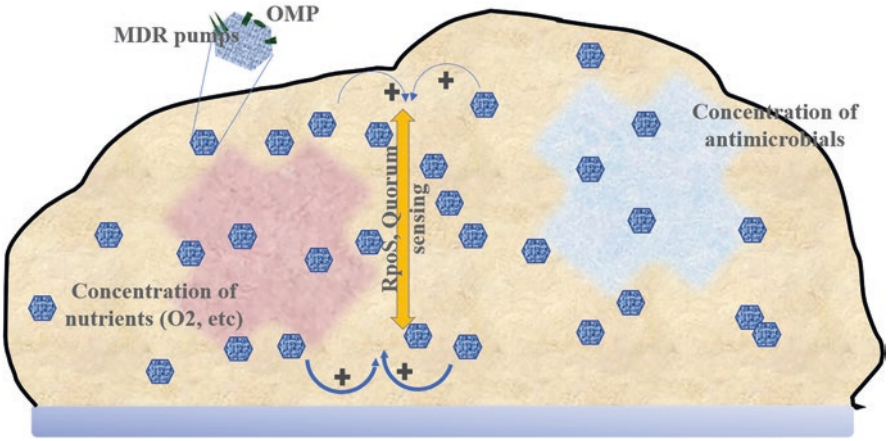

Fig. 9.2 Drug Resistance in biofilms: The yellow area stands for the extracellular polysaccharide, whereas blue polygons represent biofilm-bacteria. Nutrient gradients, oxygen and waste products can be found in biofilms, which characterize their heterogeneity (shown by colored crosses). The cellularly dense biofilm, along with its physical expulsion of many antimicrobial classes encompass resistance mechanisms. Additionally, individual biofilm-microorganisms might adapt physiologically to increase their resistance to biocides

apparent inability of some antibacterials to reach their targets in/on the biofilm cells. In the case of a mixed biofilm associated with Klebsiella pneumoniae and Ps. aeruginosa, ordinary disinfectants like chlorine were observed to reach less than 20% of the concentration present in the surrounding bulk media. This evaluation was conducted using a microelectrode specifically designed to detect chlorine levels (De Beer et al., 1994). Theoretically, penetration profile indicated that a substrate may have been consumed inside the matrix. Also, infrared spectroscopy was utilized by Suci, et al. to demonstrate that ciprofloxacin was transported to a colonized surface at a slower pace than it was to a sterile surface (Suci et al., 1994). These scientists hypothesized that the ciprofloxacin was attaching to the elements of the biofilm.

According to Larsen (2002), when *Porphyromonas gingivalis's* planktonic populations were evaluated in terms of cell density levels, comparable to those of biofilm populaces (107–108 cells mL^{-1}), the minimum inhibitory concentrations of antimicrobials (metronidazole, amoxicillin and doxycycline) were noticeably heightened. This finding suggested that an inoculum impact contributes to the enhanced bacterial resistance, but it does not fully account for the changes in susceptibilities between planktonic and biofilm organisms. Larsen's experiments also discovered the tested biofilm populaces possessed two to eight times more resistance to antimicrobials such as doxycycline and amoxicillin, as opposed to equal amounts of planktonic bacteria.

(b) *Stress responses and slow growth:* A bacterial cell culture's growth is slowed when it lacks any necessitated nutrient. Antibiotic resistance typically increases as growth goes from exponential to sluggish or no growth (Tuomanen et al., 1986a, b). In established biofilms, the bacteria have been seen to proliferate at slowed rates. It's been anticipated that this biological alteration is one of the explanations to why biofilms have resistance to many antibiotics; cells growing in biofilms are expectantly encounter varying grades of nutritional constraint.

Modern researches have explicitly explored the significance of survival of biofilm cells, with slowed growth rates, even in the presence of antibiotics. They've closely observed growth phases of biofilm-cells as well as planktonic-cells. Gilbert and colleagues (Duguid et al., 1992; Evans et al., 1991) recorded the differing growth rates of biofilms versus planktonic cultures of *E. coli*, *Ps. aeruginosa* and *Staphylococcus epidermidis*. They discovered that susceptibilities to drugs (i.e., ciprofloxacin, tobramycin) increased in parallel to the growth rate, for both biofilm and planktonic-cells. This confirmed a fundamental concept: 'biofilm-cells' slowed growth rates act as shields against active antimicrobial action'. With biofilm and planktonic forms of *Ps. aeruginosa* cells, sub-optimal growth rates yielded similar ciprofloxacin resistance. The cells in planktonic phase, however, stood more vulnerable to ciprofloxacin than the biofilm cells as the development rate was accelerated. This finding lends support to the hypothesis that the biofilm's documented resistance to antimicrobial treatment can be attributed to factors beyond its gradual formation, indicating the presence of additional contributing characteristics (Desai et al., 1998). Desai et al. conducted a comparative analysis between the resilience of biofilm and planktonic cells at multiple stages during an exponential growth phase, extending until the initiation of a stationary phase. Their investigation revealed an increasing resistance in both biofilm cells and planktonic cultures as they approached the stationary phase. The bacterial cells in biofilms had antimicrobial resistance which was 15 times more than the planktonic cells during the stationary phase of both cultures, which is when resistance was at its highest. These findings indicate that the level of resistance to certain factors is influenced by variables beyond just the growth rate of cells. It appears that delayed growth also plays a role in providing extra protection. One possible explanation for this phenomenon is the observed increase in cellular densities during later stages of exponential growth, which could be linked to this additional protective factor (Brooun et al., 2000).

(c) *Quorum sensing (QS):* Deeper comprehension pertaining to the concept of quorum-sensing (QS) in bacteria, has paved the way for advancements in knowledge on regulatory mechanisms governing drug resistance processes. The fight against drug resistance have been made possible by this discovery. Study findings demonstrate that the quorum-sensing system controls a number of cellular processes in microorganisms, including extracellular polysaccharide (EPS) synthesis, toxigenic protein production and pathogenic gene expression (Bäuerle et al., 2018; Turan et al., 2017). It also plays significant roles in the functioning of drug efflux pumps and in the development of bacterial biofilms.

Previous research of Davies and colleagues demonstrated a *Ps. aeruginosa* strain's mutation in its lasR-lasI QS system, was incapable of biofilm production with a typical structure (Davies et al., 1998). Furthermore, these authors provided evidence that lasI-mutant biofilms were abnormally susceptible to SDS treatment, albeit they did not address the issue of whether these mutant-laden biofilms had changed drug resistance (Davies et al., 1998). Bacterial efflux pump regulation by the QS system has been verified by different researches (Subhadra et al., 2019; Wang et al., 2019).

(d) *Physiologically different biofilm cells:* A developing theory in the field states that a subset of the community is given a biofilm-specific phenotype, which ignites causes active mechanisms to counter the negative effects of bactericides. Many researches of the present century are aimed at reporting activated or suppressed genes associated with biofilms and comparing planktonic-cell genes upon cellular attachment onto vulnerable surfaces (Kuchma & O'Toole, 2000). Additionally, it's feasible to say that almost all of these biofilm cells will exhibit heightened antibiotic resistance. High cell density, specific types of stress, nutritional restriction, or a combination of these factors may all contribute to the development of this resistant phenotype. Antimicrobial drugs that are unrelated chemically can be expelled from the cell through multidrug efflux pumps.

The *mar* operon is upregulated in *E. coli*, which leads to a multidrug-resistant phenotype. *AcrAB* is assumed to be the efflux pump in charge of this resistance. By using *lacZ* fusion (Maira-Litrán et al., 2000), *mar* expression was tracked in batch, chemostat and biofilm growths to look for any linkage between this well-known mechanism of multi-drug resistance and biofilm resistance to antimicrobials. *Mar* detected was lower in biofilms than it was in comparable stationary-phase culture batches, contradicting the impression that *mar* operon is elevated in biofilms.

Ps. aeruginosa has three essentially known multi-drug efflux pumps, and the *Ps. aeruginosa* genome project has also discovered numerous more putative pumps. One study made a case for the significance of one of these pumps in the development of ofloxacin resistance (Brooun et al., 2000). It demonstrated that, at lesser ofloxacin concentrations, biofilms without the pump were more responsive to this medication, than biofilms overexpressing it; *Ps. aeruginosa* strains that either lacked or overexpressed the *MexAB-OprM* pump were used. There was no change, though, for ciprofloxacin, a different quinolone. Therefore, just as with the *E. coli* investigations, more research is needed to determine whether stimulation of pumps is factually a crucial modification giving resistance to biofilms, or not.

The modification of the composition of membrane proteins due to antibiotics is another mechanism for resistance inducible in bacterial cells of biofilms. This alteration might make cells less permeable to certain substances. *E. coli* strains with mutated genes of ompB (incharge of regulation for outer membrane porin genes: OmpF, OmpC), as well as ompF, experience greater β-lactam resistance. OmpF-deficient mutants have been found to have higher levels of tetracycline and chloramphenicol resistance. Additionally, the relative ratios of the two main *E. coli* porins, (OmpC and OmpF) (Jaffe et al., 1982) were changed in starved cells, favoring the

production of OmpC, a smaller porin (Liu & Ferenci, 1998). The results mentioned above lend credence to the idea that changing porin expression alters how resistant bacteria are by nature to antimicrobials. In the past, it was also demonstrated that biofilm bacteria expressed more of the omp C gene than planktonic cells did for three other osmotically regulated genes (Pugsley & Schnaitman, 1978). These findings suggest that biofilm-microbes indeed do survive in osmotically stressful microenvironments. Therefore, a biofilm's external factors may cause changes in cell membranes, shielding the cell from the negative effects of antibacterial drugs.

Effect of Food Biofilms on Health

Many studies have shown the significance and effects of biofilms on the food industry. Biofilms showcase higher antibiotic resistance patterns than their planktonic counterparts, by up to 1000-fold, accounting for almost 20% of cases of food poisoning (Lebeaux et al., 2014). Cross-contamination between relevant food products with food pathogens, such as *Bacillus cereus*, *Campylobacter jejuni, E. coli* O157:H7, *Listeria monocytogenes, Salmonellae, Staphylococci* and *Yersinia enterocolitica,* are common (Anand et al., 2014). Apart from infections, biofilm may be associated with intoxications. For instance, in food processing units, biofilms can emit toxins, contaminating food matrices, with resultant intoxications in single or multiple persons (outbreaks). In both situations, the presence of biofilms in a food manufacturing facility poses a significant risk to public health. The level of risk associated with these persistent, complex living structures, known as biofilms, is contingent upon the specific bacterial species involved. The formation of biofilms in different types of factories is influenced by factors specific to each facility. In particular, the main areas prone to biofilm development is in proximity include: animal carcasses, assembly-line tops, contact surfaces, dispensing tubing, packing materials, pasteurizer plates, reverse osmosis membranes, storage silos for raw materials, pipelines carrying milk, water or other liquids, etc. (Colagiorgi et al., 2017).

Biofilm Microorganisms

A variety of microorganisms can flourish on foods and throughout the network of the food industry. Varying microbe species may have different capabilities for adhering to and/or forming biofilm on various surfaces. Biofilms have vastly intricate microstructures and are made up of a variety of these symbiotic microbes, including some that are pathogenic to humans, as revealed by various detection technologies and microscopic techniques. The primary microorganisms involved in the early development, colonization and spread of biofilms in the food business are discussed in this part, along with the health problems they may cause (for instance, in association with pre-prepped foods, dairies and other food matrixes) (Galié et al., 2018).

Major bacteria including, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *Enterococci*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, *Vibrio* and others, have been found to establish biofilms on foods and food-contact surfaces, raising serious questions about food safety (Sharan et al., 2022). Biofilms may be essential in ecophysiology, because they promote colonization of a variety of environmental microhabitats, such as farm-food surfaces. They may be linked to symptomless, direct colonization among some hosts, or even transmission through food systems with subsequent infection (Ahmed et al., 2013). Throughout the food business, biofilm-producing microbes may be potentially harmful to humans as they create tough complexes in manufacturing settings. These microorganisms use the processing parameters used in the food sectors, such as glass, polyethylene, stainless-steel, wood and rubber as artificial substrates (Abdallah et al., 2015; Colagiorgi et al., 2017). When disinfecting and sterilizing procedures are taken into consideration, the parameters of different microbial growth forms on foodstuffs in a processing facility involve several propensities. Choosing the best method might be challenging when attempting to prevent biofilm development in the food sector (Carrascosa et al., 2021). Table 9.1 gives brief descriptions of several of these pertinent biofilm-producing microorganisms in the food sector.

Bacillus cereus

The Gram-positive, spore-producing, facultatively anaerobic, *Bacillus cereus* (*B. cereus*), can grow in a variety of settings at temperatures ranging from 40 to 120 °F. *B. cereus* is immune to chemicals, irradiation and thermal treatments (Bottone, 2010). It is a soil-dwelling organism that is typically isolated from food and food-related goods like dairies, meats, rice and vegetables. *B. cereus* releases toxin elements which are capable of causing diarrheal food poisoning (gastroenteritis) in people (Carrascosa et al., 2021).

B. cereus-laden biofilm matrices are found on materials that come into touch with food, like storage tanks, industrial belts, machinery and metal pipes. These intricate biofilm communities are probably crucial to *B. cereus*' capacity to colonize many habitats. In conjunction with their spores, they give the microbe a high level of tolerance and adhesiveness on a number of substrates, namely stainless-steel, a substance that is of frequent use in industrial food lines. *B. cereus* can survive for extended periods of time and can endure sanitization techniques in these settings (Majed et al., 2016). They may also build up as submerged or as floating biofilms that secrete bacteriocins, proteases, lipases, metabolites and surfactants capable of altering the sensorial properties of food (Dincer et al., 2020). Their flagellar motility provide access to surfaces that are favorable for biofilm formation, and promote propagation onto uncolonized surfaces. *B. cereus* flagellar mechanisms, however, haven't been discovered to be necessarily related with adhesion to glass, but their motile activity can play a significant role in biofilm production (Houry et al., 2010).

Table 9.1 Biofilm-producing microorganisms in food sectors

Microorganism	Food contamination	Effects
Anoxybacillus flavithermus (Somerton et al., 2013)	Milk powder	Fouled skim milk powders/ products
Bacillus cereus (Bottone, 2010)	Dairies, fried rice, meats, vegetables	Diarrheal and emetic gastroenteritis
Campylobacter jejuni (Chlebicz & Śliżewska, 2018)	Poultry, raw milk	Dysentery with fever, cramps, nausea and vomiting
Clostridium perfringens (Fruin, 1977)	Pork, poultry, processed foods	Food poisoning, necrotizing enterocolitis in humans, enterotoxaemia in animals
Enterococcus species (Franz et al., 2003)	Meats	Spoilage of raw/processed meats
Escherichia coli (Galié et al., 2018)	Fruits, meats, unpasteurized milk, vegetables	Outbreaks of diarrhea, hemolytic uremic syndrome
Geobacillus stearothermophilus (Somerton et al., 2013)	Dairies	Fouled skim milk powders/ products
Listeria monocytogenes (Rothrock et al., 2017)	Cheeses and dairies, frozen vegetables, fruits, ice-creams, poultry, processed foods, raw milk	Listeriosis
Pectinatus species (Van Houdt & Michiels, 2010)	Beers	Rotten egg smell
Pseudomonas species (González-Rivas et al., 2018)	Cheeses and dairies, fruits, meats, vegetables	Reduced shelf-life of foods
Salmonella Enterica (Nguyen et al., 2014)	Cattle, fish, pork, poultry, sheep	Gastroenteritis or blood stream infection
Staphylococcus aureus (Kadariya et al., 2014)	Dairies, baked goods, egg products, meats, poultry, salads	Diarrheal/emetic gastroenteritis

Bacillus cereus is among the most common causes of gastroenteritis outbreaks, manifesting predominantly as diarrheal or emetic in nature. *B. cereus* variants associated with emetic predominance are capable of secreting non-ribosomal cyclic, heat-stable toxin peptides into food, that when consumed, causes vomiting. Simply speaking, they tolerate cooking temperatures and contribute to vomiting episodes when consumed (Ehling-Schulz et al., 2015). In accordance with the current paradigm of diarrheal *B. cereus* food poisoning, foods contaminated with spores are consumed and germination inside the gut is possible, where bacterial spores proliferate alongside the release of enterotoxins. This is particularly true for diarrheal *B. cereus* strains which secrete cytotoxin K, hemolysin BL and non-hemolytic enterotoxin (Stenfors Arnesen et al., 2008).

Campylobacter jejuni

Campylobacter species are Gram-negative, thermophilic, motile, curved bacilli, with the most common strain being *Campylobacter jejuni* (*C. jejuni*) (Klančnik et al., 2021). Aerobic as well as microaerophilic environments allow *C. jejuni* to form biofilms (Téllez, 2010). *C. jejuni* is of fastidious nature, but it can remain viable beyond the avian gut before it infects a human host. Biofilm production is triggered by a variety of external conditions, which are then influenced by a number of intrinsic variables (Tram et al., 2020).

According to the European Union One Health 2019 Zoonoses Report, *C. jejuni* is a frequent commensal in cattle, hens and turkeys. It is regarded as an opportunistic infectious agent and is considered to be a common cause of food-borne illness in humans (Authority & European Centre for Disease Prevention and Control, 2021). *C. jejuni* enters the host body via infecting and colonizing the gut, subsequently inducing food-borne illness, as a result of contaminated food processing units, water or raw milk (Chlebicz & Śliżewska, 2018).

Escherichia coli

The majority of these Gram-negative, *Escherichia coli* (*E. coli*) strains are found as non-pathogenic bacilli in the human gut microflora. The pathogenic/virulent strains consist of: enterohemorrhagic, enteroinvasive, enteropathogenic, enterotoxigenic and vero-cytotoxigenic *E. coli* (EHEC, EIEC, EPEC, ETEC, VTEC, respectively) (Gould et al., 2013). It is vital to keep in mind that a variety of *E. coli* strains have the potential to infect people, with EHEC strains being important in the food processing industry. The most prevalent from the EHEC serotype, is the O157:H7 strain, responsible for hemolytic uremic syndrome (HUS) and epidemics of bloody diarrhea around the world. They are transmissible via consumption of contaminated dark green vegetables, drinks, fruits such as melons, meats and unpasteurized milk (Galié et al., 2018; Gould et al., 2013).

E. coli's propensity to form biofilms is, in large part, what causes it to flourish widely in natural habitats. With their flagella, membrane proteins and pili, *E. coli* rods are able to attach onto non-living surfaces, creating an extracellular polymeric substance (EPS) which promotes antimicrobial and disinfectant resistance (Lim et al., 2019). Despite the fact that EHEC strains can create biofilms on a variety of surfaces involved in the industrial food sector, there isn't currently a credible way to stop *E.coli* biofilms from forming, or a better way to treat EHEC infections because using the limited available medications tends to raise the risk of renal damage and hemolytic uremic syndrome (Lee et al., 2007).

Listeria monocytogenes

Listeria monocytogenes (Gram-positive rod) is a frequently identified bacterium found in animals, decaying vegetation, food, soil, water and responsible for food-borne infection. When consumed, it can cause major difficulties in the old and young, as well as abortions in pregnant females. The infection can spread to many food forms, including cheeses, chicken, dairy goods, fish, fruits, meats, pre-packaged meals, raw milk, frozen goods including ice-creams and vegetables (Rothrock et al., 2017). The primary method of *Listeria monocytogenes* transmission to people, is through the consumption of contaminated food items (ready-to-eat food products, dairy, meats, poultry and vegetables) (Andreoletti et al., 2007).

L. monocytogenes can produce biofilm on a variety of surfaces utilized in the food business, which poses a major risk to consumer health as this could act as a source of infection. Numerous processed foodstuffs have been found to contain *L. monocytogenes*, and cooked foods can potentially become compromised as a consequence of subsequent contamination (D'Ostuni et al., 2016; Jofré et al., 2016; Vitas & Garcia-Jalon, 2004; Vongkamjan et al., 2016). *Listeria monocytogenes* can adhere to a variety of surfaces that come into contact with food, including glass, polystyrene and stainless-steel (Di Bonaventura et al., 2008). It's been discovered to linger in food sectors for decades, where it may frequently cross-contaminate food stuffs (Ferreira et al., 2014).

The pathogen can exist in complex microbial biofilms or simply as monomicrobial biofilms, and readily thrives at low temperatures (Chmielewski & Frank, 2003). *L. monocytogenes* may persist in biofilms devoid of oxygen and also can endure low-pH environments for extended periods of time. Depending on the other contending microbial population in biofilms, its population may increase or decrease (Raheem & Raheem, 2016). Most strains of *L. monocytogenes* in food industrial environments have strong adhesive abilities due to their flagellar, membrane protein and pili properties (Lemon et al., 2007).

Pseudomonas Species

Pseudomonas are heterotrophic, motile, Gram-negative bacilli which may be found as common spoiling microbes in high-pH dairy goods, on fruits, meats, vegetables, as well as in the drainage and flooring of food production facilities (Chmielewski & Frank, 2003; González-Rivas et al., 2018). These bacteria, with their extracellular filamentous extensions, have distinct effects on surface interaction and the adhesion process. Research on their flagellar and pili properties is extensive, relevant with context to biofilms, food spoilage and infections (Amina & Bensoltane, 2015). *Pseudomonas aeruginosa* (1–5 mm in length; 0.5–1 mm in width), can serve as a model bacterium for exploring the development of biofilms and how quorum sensing pathways guide them. With nitrate serving as a final electron acceptor, they are able to grow as facultative aerobes, through both anaerobic and aerobic means (Golovlev, 2002). Massive volumes of EPS are synthesized by *Pseudomonas*, which

are also known to create biofilms which adhere onto stainless steels. As part of mixed-species biofilms, they may interact with certain other bacteria, increasing their own stability and giving rise to resistance patterns (Chmielewski & Frank, 2003). On soft cheeses, *Pseudomonas fluorescens*-containing biofilms are often accompanied by pyocyanin production, a unique bluish pigment (Carrascosa et al., 2015).

Salmonella enterica

Salmonella enterica (*S. enterica*) are facultatively aerobic, flagellated, Gram-negative bacilli, which are often present as colonizers, but are also known to be associated with gastroenteritis, as well as some cases of septicemia (Lamas et al., 2018). *Salmonella*e produce curli fibers, that are proteinaceous extracellular substances associated with cell to cell and cell to surface communications (Ćwiek et al., 2019). Other than curli, many fimbriae adhesin proteins have been reported, having different biofilm-promoting properties depending on various serotypes (Grigore-Gurgu et al., 2019). In 1966, the first occurrence of complex multicellular biofilms on edible surfaces was discovered and the importance of these bacteria as a food pathogen was brought to light (Duguid et al., 1966). Food-borne salmonellosis presents as one of many common causes of food-related illness. In environments where food is handled or processed, contaminated surfaces may create biofilms that increase the danger of *Salmonella* poisoning (Corcoran et al., 2014).

From all the serotypes of *S. enterica*, the Enteritidis serovar is the one most commonly associated with febrile symptoms, abdominal pain, diarrhea, nausea and vomiting (Nguyen et al., 2014). According to a study by Russo et al., despite thorough decontamination and sterilization practices, a strain of *Salmonella enterica* subspecies *enterica*'s serotype, Agona was accountable for continuing outbreaks of food-borne illness in the microenvironments of a food manufacturing plant (Russo et al., 2013). Additionally, *Salmonella*'s Agona serovar has been responsible in the past, for recurrent outbreaks of salmonellosis (Brouard et al., 2007; Killalea et al., 1996; Russo et al., 2013; Shohat et al., 1996; Threlfall et al., 1996). The potential of different disinfection methods to limit the persistence of *Salmonella* on food surfaces was of interest in light of a significant global outbreak of *S*. Agona in 2008 (Nicolay et al., 2011). *S. enterica* strains have the potential to contaminate food streamlines, giving rise to large-scale outbreaks linked with morbidities in immunocompromised populations. It readily develops as multi-dimensional layers on stainless-steel substrates, with varying morphologies, such as reticular colonies produced when cultivated on tryptic soy broth (Wang et al., 2013). On food surfaces in industrial contexts, food poisoning-linked *Salmonella* strains speedily form biofilms, conferring *Salmonella*'s persistence in the long-run (Cogan et al., 1999; Møretrø et al., 2012; Reij & Den Aantrekker, 2004; Rodrigues et al., 2011). These biofilms may serve as reservoirs for persistent microbial contamination in food production plants, with potential to give rise to outbreaks of food poisoning (Corcoran et al., 2014).

Staphylococcus aureus

The Gram-positive coccus, *Staphylococcus aureus* (*S. aureus*), is a facultative anaerobe that can produce enterotoxins between 10 and 46 °C. *S. aureus*, when examined under the microscope, are usually visible in a cluster or grape-like arrangement. Their colonies possess a carotenoid pigment which gives them a golden color on nutrient agar, and hence the name, 'aureus,' which is Latin for 'gold' (Masalha et al., 2001).

Food handlers' mucous membranes and skin may get colonized by *S. aureus*, which can cause serious problems in food manufacturing facilities (Giaouris et al., 2015). Heat-stable enterotoxins can indeed be released alongside the bacterium, in meals accidentally contaminated by food handlers. *S. aureus* thrives well in foods containing heightened sugars or salt levels with limited water activity (Kadariya et al., 2014). Dairy, meat and poultry products provide favorable microenvironments for *S. aureus*' virulent toxin-producing strains (Adams et al., 2000). Enteric toxins produced by *S. aureus* are well recognized as having class II major histocompatibility complex-binding properties (in T-cells), increasing the risk of acute toxic shock syndrome with diarrheal illness (Schelin et al., 2017). Animals, dust, food handlers, unprocessed foods, water, etc., are known contamination sources in the food industry (Todd et al., 2009). Furthermore, it is well-established that biofilms linger onto equipment and surfaces that come into touch with food, operating as constant sources of contamination. Moreover, it was shown that factors like nutrition availability, pH, surface characteristics and temperature have an impact on subsequent microbial growth, virulence and biofilm development in this industry (Abdallah et al., 2014). According to various studies, *S. aureus* biofilms were shown to colonize food-contact surfaces in meat, poultry, dairy, pasteurization belts and seafood sectors (Gutiérrez et al., 2012; Latorre et al., 2010; Sharma & Anand, 2002).

Polysaccharide intercellular adhesins are well expressed in these cocci, and controlled by intercellular adhesion genes (ica) associated with the formation of biofilms. The fact that *S. aureus* strains lacking ica gene may still produce biofilms, nevertheless, suggests the existence of a different route, possibly the Bap (biofilm-associated protein) linked one (Toledo-Arana et al., 2005). *S. aureus*'s quorum regulator factor, SarA, is involved in positively controlling the Bap-linked pathway and increasing the transcription of ica operon (Trotonda et al., 2005).

Other Menaces and Synergisms

The non-pathogenic, Gram-positive bacillus, *Anoxybacillus flavithermus*, is a spore-forming, thermophilic, facultative anaerobe, occasionally reported as a contaminant in dairies (Strejc et al., 2020). It often presents as a challenge for sectors involved with processing of milk powders, since large concentrations will make milk powder products unfit when under food quality standards for marketing (Murphy et al., 1999). Vegetative cells of *A. flavithermus* may thrive at temperatures as high as 65 °C, and with skimmed milk there is a rise in adherent microbial cells attached

onto stainless-steel surfaces, implying that milk has a favorable impact on the development of *A. flavithermus* biofilm (Sadiq et al., 2017).

The Gram-positive rods of *Geobacillus stearothermophilus* (*G. Stearothermophilus*; also known as *Bacillus stearothermophilus*), are heat-resistant, spore-producing, facultative anaerobes (Flint et al., 2001). They develop biofilms by attaching to stainless-steel substrates on manufacturing lines, with eventual release of thermophilic colonies into manufactured products (Wu et al., 2019). Most of the biofilms found in milk or milk-product sectors worldwide, have the prevalence of thermophilic *A. flavithermus* and/or *G. stearothermophilus* strains (Burgess et al., 2009; Sadiq et al., 2017).

Gram-negative, anaerobic, *Pectinatus* bacilli have been encountered in biofilms, and linked to poor sanitation conditions in many breweries (Paradh et al., 2011). These spoilage-associated microbes were initially found in unpasteurized beer at 30 °C in a beer producing facility in Colorado (Lee et al., 1978). Since then, *Pectinatus cerevisiiphilus* species have been habitually isolated in many European breweries (Paradh et al., 2011).

Within food sectors, biofilms can be formed as a result of microbial synergisms. Certain microorganisms are capable of coexisting in food manufacturing settings, as complex microbial biofilms, from which infectious as well as spoilage microbes may contaminate edibles (Sterniša et al., 2019). For example, heterogeneous pathogens (i.e., *Aeromonas hydrophila*, *S. enterica*, *L. monocytogenes, Vibrio* species, etc.) can develop biofilms on fresh seafoods in fisheries, that may cause serious adverse consequences on both, the economy and health (Mizan et al., 2015). *Burkholderia* caryophylli, *E. coli* and *Ralstonia insidiosa*, have been found to have synergistic interactions, giving rise to tough poly-microbial biofilms in fresh-cut production facilities (Lee et al., 2007). Acylases and acyl-homoserine lactones in microorganisms, help regulate the development of polymicrobial biofilms (Lee et al., 2007).

Microorganisms adhere onto surfaces (abiotic or biotic) and utilize quorum sensing mechanisms. These mechanisms promote better cellular integration of biofilms and their dispersion via cell-to-cell communication signals (Toushik et al., 2020). Quorum sensing-regulated exopolysaccharide production as is with biofilm-forming strains of *Vibrio cholera*, has further supported the notion that cell signaling is essential for the development of bacterial biofilms (González-Rivas et al., 2018).

Numerous scientists have extensively analyzed and contrasted biofilm developmental stages of multiple microbe communities, with those of each microbe under single-microbial biofilm settings. They have discovered evidence of synergistic properties amongst certain pathogens. By examining poly-microbial biofilms, such as those containing *Candida albicans* strains, different researchers have discovered beneficial synergies in various investigations (Pammi et al., 2013; Zupančič et al., 2018). Researchers have observed that *Acinetobacter junii* and *Pseudomonas aeruginosa* found on various materials, serve as superior biofilm builders, including their attachment-deficient strains, which are capable of increasing biofilm development in commonly tested microbe communities.

Consequences of biofilms, in terms of corrosion of metals, lipase or protease-directed modification of organoleptic qualities and pathogenicity are extremely significant throughout the industrial food sector. Butter homogenizers, cheese tanks, packaging machines, pasteurizers, food belts, pipelines and raw milk stores, for instance, can serve as contact substrates for bacterial biofilm matrixes at varying temperatures and include a variety of heterogeneous colonizers and pathogens. As a result, it is crucial to establish precise techniques for detecting biofilms in situ in order to safeguard against contamination and guarantee the quality of foods.

Detection and Monitoring Techniques

Standard Methods

Traditional platforms use macro, as well as micro-scale reactors (with flow cells or static cells), to measure the development and growth of biofilms. Multi-well plates and other static instruments are frequently used in microbiology labs (Melo et al., 2012). However, the inability to consistently refill the culture media over time may cause planktonic cells along with unwanted remnants to build up, hindering any ongoing, active measurement. Alternatively, flow cells, in the form of Robbin's device (Kharazmi et al., 1999), the Calgary device (Ceri et al., 1999) and the Centers for Disease Control biofilm reactor (Goeres et al., 2005), enable only end-point disruptive analysis while providing more repeatable and controlled biofilm formation. These traditional systems can be scaled down to reduce some of their drawbacks and can be combined with brand-new sensor technologies. These innovations include portability, high-output analysis, probability, smaller test volumes and the capacity to conduct nondestructive, real-time biofilm descriptions. These traits can be used to obtain new understanding of biofilm development, interactions amongst biofilm cells and identify any likely antibiotic resistance processes taking place in tested biofilms (Meyer et al., 2011; Paredes et al., 2014a).

Additionally, with great repeatability and throughput, microfluidic devices have been used to investigate how factors like pH, flow rate, and temperature affect the production of biofilms (Gashti et al., 2016; Pousti et al., 2018). They give researchers the chance to examine biofilms in carefully regulated micro-settings, so as to replicate natural settings or in-vivo circumstances (Shumi et al., 2010). Although these systems can occasionally function independently, they frequently require integration with established biofilm tests that often use microscopy, semi-quantification (colony forming units), crystal violet staining, or other such experimentations. Microfluidic designs for nondisruptive analysis in real-time framework for biofilm testing are very limited, since these approaches demand labelling of agents, sample treatment and intrusive procedures with optical corrections (Subramanian et al., 2020). For instance, microscopy-based approaches require ongoing lens focus correction in order to record changes in biofilm depth.

Sensing Devices Advancements in sensing technologies, such as optical, electrochemical, and mechanical transducers, have facilitated the non-invasive evaluation of microbial biofilms. The high detection sensitivity of optical detection systems, for instance, comes at the expense of lengthy data collecting and analysis (Pu et al., 2021). Confocal reflection microscopy (CRM) (Yawata et al., 2010), SR-FTIR—synchrotron radiation-based Fourier transform infrared (Holman et al., 2009; Keirsse et al., 2003), white-light interferometry, Brillouin spectroscopy, SPR—surface plasmon resonance, LSPR—localized surface plasmon resonance (LSPR), fiber optics and spectro-microscopy are a few examples (Zhong et al., 2016, 2019).

Electrochemical sensors also partake biofilm testing (Clark & Lyons, 1962). Some detection methods come under the umbrella of 'impedance microbiology', built on the impedimetric sensing principle, i.e., the use of impedance variations amongst exposed electrodes to determine any involved bacteria (Firstenberg-Eden & Eden, 1984). Using these methods, researchers have been able to monitor changes in the capacitance of bacterial inoculants, conductance or impedance in order to track bacterial development in real time (Blanco-Cabra et al., 2021; Bruchmann et al., 2015; Jain et al., 2021; Liu et al., 2018; Paredes et al., 2013, 2014a, b; Pavanello et al., 2011; Poma et al., 2021; Robb et al., 2018; Ward et al., 2014; Zheng et al., 2013). In a nutshell, the biofilm-bacteria along with its extracellular elements are of dielectric nature, influential to the micro-system's overall impedance (Paredes et al., 2014a). Therefore, the development of biofilms, including any subsequent metabolic or compositional alterations within, can be inferred by tracking the impedance in the bacterial solution over time. Amperometry and potentiometry based electrochemical devices are based on measuring in terms of faradaic current generated by biofilms' redox species when the tested biofilms are laden onto electrode surfaces. These methods possess the beneficial capacity to record not only the early cell adhesion phases (Bayoudh et al., 2008; Palmer et al., 2007), but also the electroactive metabolites (Bellin et al., 2016), henceforth, monitoring specific bacterial biochemical activities on a real-time basis (Saccomano et al., 2021).

Interfacial rheometric devices, QCM—quartz crystal microbalances, quartz tuning fork oscillators, quartz tuning fork sensors, SAW—surface acoustic wave sensors and tensiometer-based devices, are also amongst the extensive list of biofilm detecting devices (de Wouters et al., 2015; Hollenbeck et al., 2014; Rühs et al., 2013, 2014). When organic or inorganic speciated cells (biofilm components) are adsorbed, surface-bound, and/or deposited onto exclusive device surfaces containing piezoelectric factors, electrical responses are generated, which are recorded accordingly. These devices are employed to real-time, with high temporal resolution and very inexpensively capture the growth of biofilms. Additionally, certain QCM-D systems are available, which are QCM-systems with added permittance of dissipation monitoring (Ripa et al., 2020). These offers additional details on mechanical characteristics of biofilm adherence, and are essential when improving eradication processes or creating surfaces that are bacterial repellant in food processing facilities or other settings. Bacterial adhesion properties and adsorption kinetics pertaining to specific surfaces may be inferred by recording mechanical responses. These

responses may be measured in terms of ‘interfacial tension’ and ‘interfacial elasticity’ of adsorbed biofilm-cells on a test surface, by means of interfacial rheometric devices, or by tensiometer-based devices (de Wouters et al., 2015; Hollenbeck et al., 2014; Rühs et al., 2013, 2014).

Emerging Methods

For fully comprehending geographic and temporal dynamics of certain metabolites in biofilms, newer detecting methods are also being explored (Saccomano et al., 2021). High variabilities in phenotypic properties of involved biofilm bacteria within the biofilm, EPS matrix structure as well as biochemical heterogeneities and the generally heterogeneousness of different microbial biofilms, all contribute to inherent challenges. Additionally, biofilm activities can be quickly impacted by intrusive procedures used to evaluate the features of the EPS matrix, microbial cells and their densities (Gloag et al., 2020). As an illustration, microelectrode probes have occasionally been used to pierce the biofilm, disrupting its structural integrity and changing the permeability of the cells (McLean et al., 2008; Peter Revsbech, 2005).

As part of some detection procedures, test biofilms are often pretreated with chemicals. This can be hindering, because, for example, when a biofilm is subjected to particular dyes, its microbial cell components may undergo biophysical and/or biochemical modifications. The challenges mentioned above encourage scientists to create less invasive methods, such as those based on pH or oxygen level trackers throughout biofilm depths. Electrical and microelectrode sensory apparatuses partake in monitoring biofilm pH, oxygen levels, ions (such as ammonium, nitrite, and nitrate), glucose, temperature changes, Ca2+ concentrations and other variables. Although these sensors are reliable and adaptable, interference or cross-sensitivity can have an impact (e.g., pH and temperature) (Wei et al., 2019). Additionally, as electrical sensors frequently have to impale through biofilms, this can decrease the measuring repeatability and alter its accuracy.

Planar optodes, or polymeric films implanted with oxygen-sensitive, luminous probes (Wolfbeis, 2015), as well as labelled micro- and nanoparticles, have been employed in optical imaging systems centered on fluorescence and confocal microscopies to monitor changes in pH, oxygen and other parameters in biofilms. Planar optodes, which allow for biofilms’ cellular attachments onto two-dimensioned polymeric films, are unable, however, to reveal detailed information on inside mechanisms of test biofilms (Glud et al., 1998; Khosravi et al., 2020; Kühl et al., 2007; Staal et al., 2011). In contrast, biofilms can be subjected to nanoparticles and microparticles laced with oxygen or pH-sensitive luminescing or fluorescing dyes. These can be distributed into EPS matrices to provide in-depth three-dimensional mapping of tested parameters (e.g., oxygen concentration, pH levels) inside the entire biofilm. In-situ biofilm studies can be conducted with less stress thanks to their nondestructive nature (Acosta et al., 2009; Jewell et al., 2019; Sønderholm et al., 2018). The detection of heavy metal adsorption (Fe3+, Cu2+, Zn2+, and

Hg2+) by biofilm microbes is pertinent to bioremediation strategies, which may be carried out with specialized fluorescent probes. C-di-GMP—cyclic di-GMP, glucose and HSL—N-acyl homoserine lactone, have drawn the most interest among the biofilm metabolites. Since glucose is the main carbon and energy source for microorganisms, its consumption directly equates with evidence of any active metabolism taking place in a biofilm. As a result, glucose has been regularly identified using conventional electrochemical sensors. C-di-GMP and HLS are significant signaling proteins (Cronenberg & van den Heuvel, 1991; Horn & Hempel, 1997). In some studies, c-di-GMPn in biofilms has been detected with electrochemical, fluorescence or luminescence-based sensors (Wang et al., 2016; Xie et al., 2015; Dippel et al., 2018). Das et al. has quantified test biofilm HLS levels using fluorescence-based sensors and Struss et al. has used colorimetric sensors for the same (Das et al., 2018; Struss et al., 2010). Dynamics of certain parameters (such as ions, metabolite products, oxygen levels, pH, etc.) within microbial biofilms have been tracked using a variety of sensing techniques.

The choice of the most suitable method for a specific application relies on a multitude of variables, including target preference and the resistance levels to intrusive procedures. For example, when aiming for high sensitivity and reliability, electrochemical sensors utilizing microelectrodes prove to be highly effective. Obtaining measurements from biofilms typically involves direct contact and penetration of the biofilm, which may pose a risk to its structural integrity. On the other hand, optical techniques that employ planar optodes and minute particles offer a less intrusive alternative for conducting such measurements. However, due to the toxic potential of the applied chemicals/dyes on microorganisms, caution is advised. Interfacial tension and elasticity-based apparatuses are accurate predictors of microbe adsorption mechanisms and biofilm development in terms of mechanical systems (Rühs et al., 2014). To be more precise, microbe adsorption at a hydrophobic interface brings about reduced interfacial tension along with increased interfacial elasticity. Rheological and tensiometric techniques can be used to measure these material properties, which can then be connected with bacterial adhesion traits.

Methods for Detecting Biofilms on Food Surfaces

There are two types of biofilm evaluation approaches in use (Fig. 9.3): direct and indirect. Examples of direct methods are: enzymatic reaction tests, contact plates, laser scanning confocal, epifluorescence microscopy, scanning electron microscopy (SEM), atomic force microscopy, environmental-SEM, cryo-SEM, focused ion beam-SEM. These allow direct observance of associated biofilm microcolonies and their involved bacteria (Van Houdt & Michiels, 2010). The foundation of indirect approaches is the separation of involved bacteria from their contact interfaces, prior to quantifying them. They consist of methods for determining the viability of microorganisms, namely: conventional plate counting tests like TEMPOR (bioM'erieux, Marcy l'Etoile, France), methods for measuring impedance, staining biofilm biomass using safranin or crystal violet and performing metabolite tests (XTT assay,

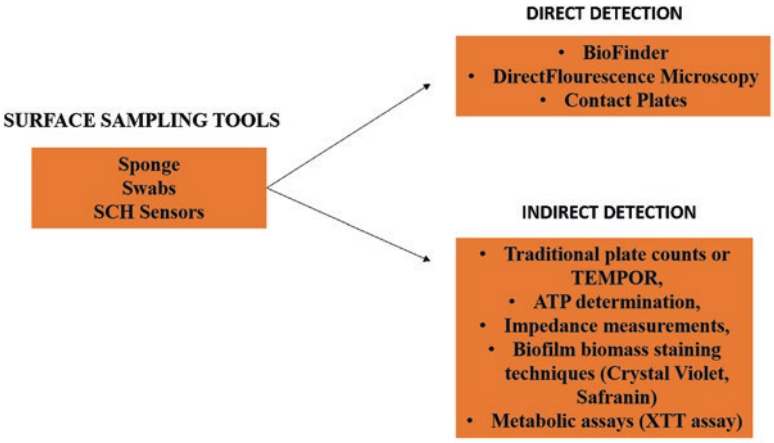

Fig. 9.3 Methods for detecting biofilms on food-contact surfaces and sample surfaces

Alamar Blue assay) (Verran, 2002). Though each method has benefits as well as drawbacks, merging several techniques ensures improved detection (Van Houdt & Michiels, 2010).

Biofilm Disrupting Technologies and Techniques

Bacteriocins

Bacteriocins are proteinaceous or peptide toxins normally produced by bacteria to prevent any related bacterial strains around them. Around a 100 years ago (1925), Andre Gratia discovered bacteriocins (Gratia, 2000). Bacteriocins are commonly synthesized as non-biological active precursor peptides possessing N-terminal leader sequence; however, in some cases, precursors undergo post-translational modification (Soltani et al., 2021) . The ensuing molecular event may lead to the cleavage of the leader region and the export outside the cell (Mokoena, 2017). The generated antimicrobial peptides exhibit bactericidal or bacteriostatic properties directed against the closely related strain (Hatakka et al., 2008). The beauty of bacteriocin-producing cells is that they set up a mechanism to prevent them from being harmed or destroyed by their own toxins. For self-defense, they may produce immunomodulatory proteins, utilizing efflux pumps or a combination of both systems to stay safe (Bastos et al., 2015).

Classification of Bacteriocins

Bacteriocins are classified based on strain, resistance mechanism, and killing mechanism.

Recently, Cotter et al. categorized the bacteriocins of both Gram-positive and Gram-negative bacteria (Cotter et al., 2013).

Bacteriophages

Bacteriophages, commonly known as phages, typically infect and replicate in bacterial cells. Bacteriophages are viruses that exist widely throughout the environment and are recognized as the most abundant biological entities on Earth (Schmaljohn & McClain, 1996). Genome-wise, bacteriophages are exceptionally diverse, containing proteins that encapsulate DNA/RNA. They may encode a few genes (MS2) or several hundred genes based on genome size. Bacteriophages display a species-specific nature concerning their hosts, primarily infecting single bacterial strains or particular species. Replication of phages occurs within the bacterium following the introduction of their genome into the cytoplasm.

The resistance of biofilms to bacteriophages is attributed to the impermeability of the biofilm matrix, which hinders the phages' ability to penetrate and effectively target the bacterial cells within the biofilm. It is a well-known fact that the genome of bacteriophages possesses genes of enzymes capable of breaking down the matrix of biofilm (Leiman et al., 2009; Sillankorva et al., 2011). Sometimes soluble enzymes usually target the host bacterial cell wall and release the host cell. These enzymes have the potential to degrade the biofilm extracellular polymeric substance while releasing it from the lysed host cell. During the process, cells also liberate DNA, and DNAses may contribute to the biofilm matrix. Bacteriophages like T4 and HK260 (bacteriophages of *E. coli*) encompass an enzyme on the tail of the virus particle, through which they penetrate the bacterial cell wall. The enzyme may play an important role in degrading the biofilm matrix and is often concealed until the tail reconfigures during and after infection and impart localized action (Leiman et al., 2004).

These proteins are specific as they fit within the virus structure and function accordingly. Yan et al. presented a 'common model bacteriophage (tailed) infection'. Here, constituents of the tail first recognize and then digest the capsular polysaccharide. The tail penetrates the cell membrane and injects the bacterial genome inside. The tail structure of bacteriophage usually possesses the polysaccharide depolymerase protein, and tail spike protein has endoglycosidase activity which hydrolyses the polysaccharide receptors.

Bacteriophages possess the ability to induce gene expression of depolymerase enzyme in the host bacteria (Topka-Bielecka et al., 2021).

Phage-based management is currently being exploited to combat biofilm via numerous mechanisms. Previously it has been reported that phages are strictly species-specific viruses where they infect bacteria and are totally dependent upon the host during the replication process. Recently, with the emergence of antimicrobial resistance and subsequent discoveries of new antibiotics, phage and phage therapy have been brought into mainstream research to combat the new species of bacteria. Several thousand phages have been discovered recently (Ackermann &

Prangishvili, 2012). Based on basic structural forms, phages are classified into four basic categories, namely (i) tailed phages, (ii) polyhedral phages, (iii) filamentous phages, and (iv) pleomorphic phages (Basic Phage Electron Microscopy | Springer Nature Experiments, n.d.). The interaction between phage and host cells relies entirely upon the receptor-binding protein present in the tail fiber of phages (Zinke et al., 2022).

Two enzymes mainly responsible for the antibacterial activity of phages are depolymerases and lysins. Depolymerases degrade capsular polysaccharides, while lysins destroy peptidoglycan present in the cell wall of bacteria (Schmelcher et al., 2012). At the tip of phage (tail fibers), the domain of depolymerase is frequently exhibited; however, the lysins are encoded either on the tail or inside of virion particles and can cut the peptidoglycan of cell wall from outside or inside respectively (Sharma et al., 2017).

Lysins Phage lysins, depending on their target bacteria, are categorized into two types: Gram-positive or Gram-negative lysins. These lysins are hydrolytic enzymes that are produced towards the end of the phage's lytic replication cycle. Their main function is to cleave the bacterial cell wall from within the cell, leading to the liberation of new phage particles. Moreover, lysins can exhibit an external action by assisting in the permeation of the bacterial cell by the parental phage. In addition to their phage-related functions, lysins possess the ability to dismantle the extracellular polymeric matrix of biofilms and effectively target the bacteria residing at the periphery of the matrix. Due to the absence of an outer membrane (OM) in Gram-positive bacteria, lysins work efficiently, while in Gram-negative bacteria, OM hampers lysin penetration to reach the peptidoglycan.

However, recent research has presented that four Gram-negative bacteria target endolysins (LysAm24, LysAp22, LysECD7, and LysSi3) exhibiting antibacterial activity *in vitro* as well as *in vivo*.

In the case of Gram-positive lysins, the cell binding domain (CBD) harboring at C-terminus is accountable for interacting with the cell wall, while the enzymatically active domain (EAD) present on the N-terminus responsible for the hydrolysis of peptidoglycan. Gram-negative lysin does not interfere with CBD and typically employs a globular conformation having a single EAD to hinder bacterial cell walls (Becker et al., 2008). Currently, lysins are manipulated as free enzymes as an alternative to antibacterial drugs in treating biofilms. Lysins have also been used extensively in the multi-drug resistant *S. aureus* in clinical settings. Chimeric lysins such as ClyH and ClyF have shown a large percentage of biofilm mass reduction (Yang et al., 2014, 2017).

Depolymerases Depolymerases are the class of enzymes that interfere with and degrade the capsular polysaccharide of Gram-negative bacteria. Depolymerase is generally encoded as a portion of phage structure. Various known depolymerases work against a range of bacterial species and have been recently employed for biofilm destruction. Phage depolymerases have further subdivided into two groups based on different mechanisms: hydrolases and lyases (Knecht et al., 2019).

Hydrolases function in cleaving the substrates, which utilizes hydrolysis with the involvement of water (Knecht et al., 2020). In the previous section, we discussed the main component of biofilm, i.e., EPS, which forms 50–90% of the total biofilm organic component and thereby can interfere with biofilm formation (Flemming & Wingender, 2010).

Depolymerases derived from phage may demonstrate two approaches against antibiofilm management, namely (i) free enzyme and (ii) tail spike protein (TSP). Free depolymerase shows a certain degree of advantage over TSP as it provides extended molecular stability, efficiently delivers via diffusion and diminishes chances of resistance development (Chen et al., 2022). Depolymerase (Dpo42) extracted from the ORF42 of the vB_EcoM_ECOO78 E. coli phage. After subsequent purification and expression via *E. coli* BL21 as a free protein, it was determined that Dpo42 successfully degraded the capsular polysaccharide encompassing the *E. coli* and prevented biofilm formation (Guo et al., 2017). The specificity of depolymerases is that it degrades bacterial capsules and the glycocalyx, the main constituent of biofilm (Chan & Abedon, 2015).

TSP depolymerase opened the vista for medical device application. Recent studies on *A. baumannii*-adhered catheters have shown effective inhibition of bacteria within a few hours (4 h) of treatment (Shahed-Al-Mahmud et al., 2021). It was also found that TSP derived from φAB6 may postulate potential management against MDR *A. baumannii* infection in the next decade.

Phages possess the inherent capability to destroy bacterial hosts and thereby inhibit the formation of biofilm (Domingo-Calap & Delgado-Martínez, 2018). The existing biofilm can also be penetrated by phages and destroy the biofilm structure with or without killing the resident bacteria.

Broadly, biofilm disruption with the application of phages has been cand be divided into types, namely (Chan & Abedon, 2015):

- extra- to intra- cellular degradation of bacterial structure
- intra- to extra- cellular degradation of bacterial structure
- chemical dispersion of biofilm matrix—particularly of EPS

Biosurfactants

Biosurfactants prevent biofilm formation by various mechanisms like (i) changing the cell adhesion capability, (ii) membrane disruption, and (iii) inhibiting the electron transport chain (Satpute et al., 2016). Biosurfactants are microorganism specific and exhibit antifungal, antibacterial, and antibiofilm activities depending upon the species (Paraszkiewicz et al., 2021). Biosurfactants decrease the growth of biofilm produced by *S. aureus* by controlling the expression of genes like dltB, cidA, and icaA (Yan et al., 2018). A significant reduction of gene expression of cidA gene was shown from the biosurfactants obtained from *Lactobacillus plantarum* at a concentration of 12.5 mg/mL (Yan et al., 2018). Similarly, biosurfactants obtained from

Pediococcus acidilactiti at a concentration of 50 mg/mL affect gene expression by downregulating autoinducer-2 signaling molecules, accessory gene regulator, and staphylococcal accessory regulator (Yan et al., 2018). Liposome-derived *Lactobacillus*-based biosurfactants exhibit more inhibition of *S. aureus* biofilm formation and elimination as compared to free biosurfactants (Giordani et al., 2019).

Recently identified lipopeptides from *Acinetobacter junii* capable of self-aggregate to form sheet rich biosurfactant vesicle having thermostable properties and less toxic are utilized as promising antibiofilm agents (Ohadi et al., 2020). Another lipopeptide biosurfactant isolated from *Bauveria bassiana* significantly removes biofilm in ex vivo surroundings for *M. canis* (Abdul-Aziz et al., 2020). Here, biosurfactants disturb the integrity of the cell membrane and affect cell membrane permeability. Inexpensive biosurfactants of *B. bassiana* are normally produced from steep corn liquor and are widely used against recalcitrant dermatophytosis. Surfactin (cyclic lipopeptide) was reported to have promising results against *C. albicans* biofilm-associated infections. Surfactin controls the expression of several genes required for hyphae production and acts by reducing the surface hydrophobicity of cells (Janek et al., 2020).

Rhamnolipids obtained from *Pseudomonas aeruginosa* MN1 possess higher antibiofilm and antiadhesive properties compared to Surfactin (Abdollahi et al., 2020). Glycolipid fabricated from *Burkhoderia* sp. WYAT7, an endophyte of *Artemisia nilagirica* (Clarke) Pamp, shows antibiofilm activities versus *S. aureus* (Ashitha et al., 2020). Glycolipoprotein, rich in LeuHis- Trp amino acids isolated from *Acinetobacter indicus* M6, may remove more than 80% of biofilm at a concentration of 500 μg/mL (Karlapudi et al., 2020).

Biosurfactants are exploited as a coating agent for medical devices such as urinal catheters and bone implants to prevent biofilm formation from the pathogenic organism. Rhamnolipids and sorphorolipids hinder biofilm formed by Gram-positive and Gram-negative bacteria (Sharma et al., 2021). Biosurfactants isolated from *Lactobacillus acidophilus* restrict biofilm generation of *S. aureus* and *Proteus vulgaris* on polydimethylsiloxane-based implants (Satpute et al., 2016).

Blockage of Quorum Sensing

Quorum sensing (QS) is the phenomenon to detect, respond, and communicate within the bacterial community by regulating gene expression. QS plays a significant role in the regulation of diverse cellular properties in bacteria, such as bioluminescence, antibiotic resistance, virulence gene expression, and biofilm formation (Li et al., 2012). QS is an efficient strategy to restrict biofilm formation where cell-cell communication stops (Chen et al., 2022; Sharma et al., 2021).

QS system is broadly divided into three main categories (Brackman & Coenye, 2015):

- Acyl homoserine lactone—i.e., AHL (Gram-negative organisms)
- Autoinducing peptide—i.e., AIP (Gram-positive organisms)
- Autoinducer-2—i.e., AI-2 (Gram-staining bacteria)

Homoserine lactones are an important class of cellular signaling molecules implicated in QS and AHL-dependent QS primarily exhibited by Gram-negative bacteria (Li & Tian, 2012). Interestingly, AHLs are produced by particular cognate AHL synthetase, and increasing concentration of AHLs are correlated with substantial growth of bacteria. AIPs are also signaling molecules synthesized by Gram-positive bacteria and secreted by membrane transporters. Once the concentration of AIPs increases in the bacteria, they interact with histidine kinase sensors and phosphorylates. Due to phosphorylation, gene expression takes place and is strictly regulated by an accessory gene regulator (agr), which is associated with the secretion of AIPs.

Potential Anti-biofilm Nanotechnologies

Chemical Processes

There are various chemicals that can disrupt biofilms, and they can be broadly categorized into enzymes, surfactants, quorum sensing inhibitors, and antimicrobial agents. Enzymes such as DNase, protease, and dispersin B can degrade the extracellular matrix components of biofilms and weaken their structure. Surfactants such as sodium dodecyl sulfate (SDS) can penetrate the biofilm and disrupt the cell membrane, leading to cell death and biofilm disruption (Flemming & Wingender, 2010; Kaplan, 2010; Vasilev et al., 2009). Quorum sensing inhibitors (QSIs) are chemicals that can interfere with the cell-to-cell communication mechanism of bacteria, which is essential for biofilm formation (Wu et al., 2015). Examples of QSIs include furanones, halogenated furanones, and azithromycin. These compounds can disrupt biofilms by preventing the production of extracellular polymeric substances and inhibiting cell adhesion (Tateda et al., 2003). Antimicrobial agents such as antibiotics and biocides can also disrupt biofilms. However, their efficacy is often limited by the biofilm's ability to create a protective barrier that reduces their penetration and neutralizes their effect. Therefore, higher concentrations of antimicrobial agents are required to disrupt biofilms compared to planktonic bacteria (Mah & O'Toole, 2001).

Overall, the disruption of biofilms by chemicals is a complex and challenging task, and the choice of the appropriate chemical will depend on the type of biofilm and the specific microorganisms involved.

Enzymatic Interference

Enzymes are a type of protein that can interact with non-protein molecules called cofactors, and they have the ability to accelerate the speed of chemical reactions in biological systems. In other words, they act as catalysts, facilitating reactions without being consumed in the process.

The eradication of biofilms typically necessitates rigorous mechanical, physical, or chemical interventions, which may not be viable for delicate medical equipment, such as endoscopes, rendering them vulnerable to bacterial colonization (Stiefel et al., 2016). Moreover, the application of harsh methods is not always feasible for eliminating biofilms caused by pathogens within the human body, thereby contributing to the persistence of infections, chronic wounds, and malfunctioning medical devices (Del Pozo, 2018; Metcalf & Bowler, 2013).

Therefore, enzymes can be utilized as an alternative to chemical and mechanical means for the dispersion of biofilms under mild conditions, such as physiological temperatures. Through enzymatic treatment, biofilms on tank and pipe surfaces can be effectively removed by breaking down the essential components of the biofilm matrix (Lequette et al., 2010; Simões et al., 2010). These enzymes are designed to specifically target the primary constituents of biofilms, which include exopolysaccharides, proteins, and nucleic acids. Enzymes work by breaking down the various structures that make up the extracellular polymeric substance (EPS) of a biofilm, which ultimately results in a reduction in the biofilm's physical integrity. To achieve an effective removal of the biofilm, it is crucial to first identify the specific structural components of the EPS before applying the enzymes (Molobela et al., 2010).

There exist four distinct classes of enzymes that are commonly employed for the purpose of eliminating biofilms. These enzyme types include proteolytic enzymes, which target proteins, polysaccharide-degrading enzymes, which break down complex carbohydrates, oxidative enzymes, which trigger oxidation reactions, and anti-quorum sensing enzymes, which inhibit the signalling mechanisms utilized by biofilm-forming bacteria (Bzdrenga et al., 2017; Johansen et al., 1997; Thallinger et al., 2013).

Essential Oils

Essential oils are volatile and aromatic compounds that are derived from various parts of plants such as petals, seeds, leaves, stems, and roots through natural processes, and are considered to be their essence or fundamental nature.

Essential oils are endowed with properties that enable them to inhibit the growth of plasmodium, fungi, and bacteria (Utchariyakiat et al., 2016). It possess properties that enable them to inhibit the growth of food spoilage and foodborne pathogenic bacteria, thus making them effective as food preservatives also (Bai & Vittal, 2014). There are a variety of naturally occurring substances and medicinal plants that can be found in fruits, spices, and phytochemicals. These substances have the ability to inhibit quorum sensing, which is a process used by certain bacteria to communicate with one another and coordinate their behavior. Essentially, these natural products contain compounds that can disrupt the ability of bacteria to communicate with one another, potentially leading to a reduction in harmful bacterial activity (Adonizio et al., 2008; Sybiya Vasantha Packiavathy et al., 2012; Vandeputte et al., 2010; Vattem et al., 2007).

EOs exhibit strong antibacterial effects against both Gram-positive and Gram-negative bacteria, whether they are in a stationary or mobile state (Essential oils against bacterial isolates from cystic fibrosis patients by means of antimicrobial and unsupervised machine learning approaches | Scientific Reports, n.d.; Millezi et al., 2016). Essential oils are known for their volatile nature, which gives them the ability to produce vapor that exhibits potential antimicrobial properties. Various studies have shown that the vapor phase of essential oils such as cassia, cinnamon, cherry laurel, origanum, and thyme, possess inhibitory effects against a diverse range of bacteria. Additionally, these oils have been found to be effective in preventing the growth of molds in food products and combating bacteria that form biofilms (Benzaid et al., 2019; Ji et al., 2019; Maruzzella & Sicurella, 1960).

Measures to Block Quorum Sensing

Quorum sensing is a sophisticated mechanism used by microorganisms to communicate with each other and coordinate their behavior. This communication system enables microorganisms to sense when their population reaches a certain density or "quorum", and respond accordingly by regulating gene expression and producing specific molecules that can affect the behavior of neighbouring cells. These signaling molecules act as chemical messengers, much like hormones in higher organisms, and allow microorganisms to act in a synchronized and cooperative manner. By using quorum sensing, microorganisms are able to coordinate their activities, such as forming biofilms or carrying out group behaviours, in a way that maximizes their chances of survival and success (Bandara et al., 2012; Hawver et al., 2016; Hense et al., 2007; Redfield, 2002).

Biofilms can be formed as a result of this communication system, and these infections can be difficult to treat with antibiotics. Bacterial infections can potentially be prevented or treated by blocking quorum sensing. Numerous studies have demonstrated with convincing evidence that QS inhibitors are capable of effectively impeding the formation of biofilms (Chen et al., 2018b; Ouyang et al., 2016).

Quorum Sensing Inhibitors

These are molecules that interfere with the quorum sensing signalling pathways and prevent bacteria from communicating with each other. There are many different types of quorum sensing inhibitors, such as natural products, synthetic compounds, and peptides.

Plants Based QS Inhibitors

There exists a plethora of natural substances that can effectively inhibit biofilm formation by interfering with the process of QS. These compounds are predominantly sourced from plants. As an illustration, curcumin, a compound found in the Curcuma longa plant, has been observed to impede the development of biofilms in various uropathogens, including Pseudomonas aeruginosa PAO1, Escherichia coli, Serratia marcescens, and Proteus mirabilis. This is achieved by curcumin's ability to decrease the production of exopolysaccharide through the inhibition of quorum sensing. Moreover, curcumin also shown the ability impede bacterial motility, which further slows down the formation of biofilms (Packiavathy et al., 2014).

Likewise, the natural compound resveratrol has been found to interfere with QS signaling in P. aeruginosa PAO1 by binding to the protein receptor LasR, thereby hindering the formation of biofilms (Vasavi et al., 2017). Similarly, carvacrol was shown to inhibit biofilm formation and pyocyanin production in P. aeruginosa (Tapia-Rodriguez et al., 2019). Additionally, naturally occurring furocoumarins sourced from grapefruit have demonstrated inhibitory effects on the biofilm formation of E. coli O157:H7, P. aeruginosa, and Salmonella enterica serovar Typhimurium (Girennavar et al., 2008).

Since these nontoxic, natural, biofilm inhibitors pose no harm to environment and the host, they offer great capability for application in diverse fields.

Synthetic QS Inhibitors

Apart from natural compounds, synthetic compounds have also been identified to have the ability to inhibit QS signalling pathways.

For instance, synthetic compounds such as furanone C-30 have been studied. Furanone C-30 has been identified as an effective biofilm inhibitor in S. mutans (He et al., 2012). Similarly, 2(5H) Furanone has been found to reduce microbial motilities and biofilms of C. jejuni strains by disrupting QS activities (Castillo et al., 2015). Another synthetic compound, Meta-bromo-thiolactone, has been demonstrated to inhibit the production of pyocyanin and biofilm formation in P. aeruginosa. This inhibition is achieved through the compound's ability to bind to two QS signal receptors, namely LasR and RhlR (O'Loughlin et al., 2013).

Biofilm inhibitors that target QS pathways have been extensively utilized for inhibiting a wide range of biofilms. However, there are still numerous QS inhibitors currently under development that hold potential for treating infections caused by biofilms or eliminating biofilms that form on tissue implants (Luo et al., 2017; Yu et al., 2018).

In addition to the use of QS-based biofilm inhibitors, alternative strategies exist for controlling biofilm formation. One such approach involves combining QS inhibitors with antibiotics to achieve superior biofilm control (Thomann et al., 2016).

Quorum Quenching

As a result of the increasing prevalence of antibiotic-resistant bacteria resulting from overuse of antibiotics, it has become crucial to explore alternative methods of fighting microbial infections. One such approach is quorum quenching (QQ), which involves interfering with the process of microbial communication. By using QQ-driving molecules, it is possible to reduce or completely inhibit the production of virulence factors, such as biofilm formation. This can prevent bacterial populations from coordinating their activities and limit their ability to cause infections. Thus, QQ has emerged as a promising strategy for developing new antimicrobial therapies that can effectively combat antibiotic-resistant bacteria by targeting their communication mechanisms.

The enzymatic breakdown of AHL molecules is the most well-known mechanism of quorum quenching, and this process is facilitated by four different groups of enzymes: lactonases and acylases, which break down the HSL ring and amide bond of AHL, respectively, and reductases and oxidases, which modify the activity of AHL without fully breaking it down (Rehman & Leiknes, 2018). Inducer antagonists represent an additional mechanism for disrupting bacterial communication. These molecules can inhibit the transmission of signals between cells by either binding to the receptor in competition with inductors or by non-competitively blocking the inductor-mediated signal transmission into the cell (Bodede et al., 2018). Various approaches to quorum quenching have been identified, including inhibition of signal molecule synthesis, such as AHL, through the use of C8-HSL to impede LuxI enzymatic activity (Hirakawa & Tomita, 2013); blocking of signal transduction cascades using small molecule inhibitors like savrin, which interferes with AgrA and inhibits the production of RNAIII and virulence factors (Sully et al., 2014) and inhibition of QS signal molecules in Gram-positive bacteria through kinase inhibitors like closantel, RWJ-49815, and LY266500 (Brackman & Coenye, 2015).

Use of Anti-quorum Sensing Antibodies

AHL and AI-2 signaling activation can trigger programmed cell death by affecting the host's immune system (Gupta et al., 2011; Khajanchi et al., 2011), but researchers have discovered ways to interfere with this process using monoclonal antibodies. For instance, the RS2-1G9 antibody can bind to 3-oxo-C12-HSL in the extracellular environment of Pseudomonas aeruginosa to reduce the host's inflammatory response (Park et al., 2007), while the XYD-11G2 antibody catalyzes the hydrolysis of 3-oxo-C12-HSL signaling, inhibiting pyocyanin production by Gram-negative bacteria (Koul et al., 2016; Praneenararat et al., 2012). Additionally, the AP4-24H11 monoclonal antibody can block the QS signal of Gram-positive Staphylococcus aureus by interfering with AIP IV, which has been shown to

attenuate tissue necrosis in infected models (Grandclément et al., 2016; Park et al., 2007). Although promising, the use of these monoclonal antibodies for treating bacterial diseases is still in the early stages.

Non-thermal Plasma

In settings where nosocomial biofilms need to be removed, traditional methods such as high heat and chemical exposure may not be ideal due to the potential for surface damage and environmental contamination with toxic chemicals. However, a promising alternative technique, called non-thermal plasma (NTP), has the potential to effectively decontaminate or sterilize nosocomial biofilms (Thapa & Ayan, 2019).

NTP is an emerging tool for improved biofilm sterilization (Koban et al., 2011; Thapa & Ayan, 2019). Plasma, the fourth fundamental state of matter, contains free radicals, reactive oxygen and nitrogen species (Jha et al., 2017), and positive and negative ions (Gaunt et al., 2006; Graves, 2012), which act as potential antimicrobial agents. Two distinct types of plasma, namely thermal and non-thermal, can be distinguished based on the relative energy levels of electrons and heavy particles they contain (Moreau et al., 2008), Thermal plasma is characterized by having both electrons and heavy particles at the same temperature, which is achieved through high pressure and power conditions. On the other hand, NTP consists of electrons at higher temperatures while heavy particles remain at room temperature. This state is produced under low-pressure and low-power conditions (Hoffmann et al., 2013; Moreau et al., 2008).

Thermal plasma has been utilized for purposes such as tissue removal, sterilization, and cauterization. However, the high heat production associated with thermal plasma can result in tissue and surface damage. On the other hand, NTP such as DBD and jet plasmas can carry out the same functions without causing harm or side effects (Keidar et al., 2013), making it suitable for biological and medical applications. Recent studies have revealed encouraging outcomes regarding the sterilization and decontamination of biofilms formed by various bacterial species using NTP (Ayan, 2009).

There are non-thermal jet plasma devices that use atmospheric pressure plasma, which are available for commercial use (Weltmann et al., 2009). One such device is the kINPen®, designed for biomedical applications, that allows for precise and arbitrary movements in three dimensions (Bekeschus et al., 2016). Applying a high-frequency voltage to the pin-type electrode generates the plasma, which is considered electrically safe as it is certified and compliant with EU standards (Weltmann et al., 2009). kINPen® plasma, which primarily uses argon gas but can also use other gases in smaller amount (Reuter et al., 2015), is a safe and effective medical device for antimicrobial purposes and wound healing, as demonstrated by clinical studies on both animals and humans. Its predecessor, kINPen®MED, was the first atmospheric pressure plasma jet device to receive accreditation as a medical device for patient use (Bekeschus et al., 2016).

Only few other plasma sources, including SteriPlas (AdTec Ltd., Japan), PlasmaDerm (Cinogy GmbH, Duderstadt, Germany), and Plasma One (Medical Systems GmbH, Bad Ems, Germany) (Bekeschus et al., 2016), have been certified as medical devices, and they have been used for various biomedical applications such as wound healing, chronic leg ulcers (Brehmer et al., 2015; Heinlin et al., 2013), reducing bacterial populations in wounds (Isbary et al., 2010), and biofilm decontamination or sterilization (Thapa & Ayan, 2019).

Coating Surfaces

Applying a coating to the surfaces where microbial attachment occurs can serve as an effective strategy to prevent the adherence of microorganisms and the subsequent formation of biofilms.

The wettability of a surface, which is determined by its surface free energy (SFE), can have a significant impact on the attachment of microorganisms. Microbes can attach to surfaces by forming biofilms, and surfaces with high SFE are more hydrophilic and thus more attractive to microbes for attachment (Nakamura et al., 2016).

However, other factors such as surface roughness, charge, and chemistry can also affect microbial attachment. Therefore, considering the SFE and wettability of a surface is important when designing materials to prevent microbial attachment, especially in fields like medical devices, food processing equipment, and water treatment systems.

A research scenario involves modifying the SFE of denture materials by applying salivary and/or blood plasma proteins. This alteration can effectively hinder the attachment of Candida albicans and prevent the formation of biofilms (da Silva et al., 2015). Microorganisms face challenges in establishing colonies on surfaces that exhibit superhydrophilic properties (Almaguer-Flores et al., 2012).

The application of a coating consisting of small molecules has the potential to modify the adhesive properties of the underlying surface materials. One way to modify the properties of silicone rubber surfaces, which are commonly utilized for creating tissue implants, is by applying a thin layer of a chemical mixture called monomeric trimethylsilane (TMS)/O2. This alteration has been observed to greatly impact the way in which certain microbial surface proteins adhere to the surface, ultimately inhibiting the formation of biofilms by the bacteria S. aureus (Xu et al., 2015). The TMS/O2 coating technique is a highly effective and eco-friendly method, with significant potential for use in various clinical applications.

The use of an antimicrobial peptide coating represents a valuable approach to prevent the formation of biofilms. It has been discovered that titanium discs, which are chemically bonded with GL13K, demonstrate remarkable antimicrobial properties against Streptococcus gordonii. Additionally, the coating effectively prevents the attachment of S. gordonii to the treated surface (Chen et al., 2018a) GL13K, a cationic peptide with bactericidal properties derived from BPIFA2, a secretory

protein produced by the parotid gland in humans (Hirt & Gorr, 2013), as a coating on titanium surfaces, has been found to be highly effective in reducing the growth of two types of bacteria—Fusobacterium nucleatum and Porphyromonas gingivalis. Moreover, this coating also prevents the formation of biofilms by these organisms (Li et al., 2017).

Therefore, coating the surface of tissue implants and medical devices with certain materials is an effective method of preventing bacterial infections. A reduction in microbe attachment to materials, prevention of biofilm formation, and reduced risk of bacterial infections can all be achieved this way.

Potential Anti-biofilm Nanotechnologies

Due to the intricate nature of biofilm, traditional methods are unable to completely eliminate them (Chaudhary et al., 2020). Moreover, due to their resistance to antibiotics, higher therapeutic doses may be necessary, which increases the risk of systemic toxicity. Hence, researchers aim to overcome these constraints by utilizing various methods, such as nanoparticle-based drug delivery systems and interference with bacterial communication pathways using small molecules that regulate biofilm formation (Diab et al., 2015; Eleraky et al., 2020; Lopez-Leban et al., 2010; Tamilvanan et al., 2008).

Nanoparticles exhibit two primary mechanisms for better anti-biofilm properties: (1) direct interaction with single cells and (2) interaction with or denaturation of the EPS matrix. The unique properties of nanoparticles make them suitable for controlling biofilm infections. The size and shape, as well as the surface and interior properties of nanoparticles, are essential factors in the control of biofilm infections (Diab et al., 2015; Moghadas-Sharif et al., 2015; Ramos et al., 2018; Tan et al., 2020).

Nanoparticles possess unique physical and chemical properties due to their small size, which makes them an attractive research area for many fields including photochemistry, electrochemistry, and biomedicine (Haruna et al., 2016; Liu et al., 2019). Their high surface area and distinct electronic properties make them stand out from their bulk counterparts. Experts have determined that the optimal size range for Nps, which are used to control biofilm infections, is between 5 and 200 nm. It is important to note that Nps should not exceed 500 nm in size to ensure maximum effectiveness (Liu et al., 2019).

Nanoparticles have diverse therapeutic applications and can be synthesized from various inorganic and organic compounds. Inorganic materials are essential for simultaneous therapy and diagnosis due to their easy modification, high drug loading capacity, and stability (Saleh, 2014). The utilization of nanoparticles in the pharmaceutical sector, including its use in drug delivery systems, is well known (Huang et al., 2008).

Zinc Oxide Nanoparticles (ZnO-NPs)

ZnO NPs are highly effective in preventing biofilm infections due to their potent antibacterial properties (Padmavathy & Vijayaraghavan, 2008). Numerous studies have confirmed their ability to inhibit the growth of various bacteria, including P. aeruginosa (Dwivedi et al., 2014; Lee et al., 2014), S. pneumonia (Bhattacharyya et al., 2018), B. subtilis, E. coli, and P. vulgaris (Abinaya et al., 2018; Hsueh et al., 2015; Ishwarya et al., 2018). Moreover, research has also shown that ZnO-NPs can significantly reduce biofilm growth of certain fungi such as Alternaria alternate, Penicillium chrysogenum, and Penicillium pinophilum. However, their impact on Aspergillus niger was not as significant (Gambino et al., 2017).

Magnesium Oxide Nanoparticles (MgO-NPs)

MgO-NPs are a promising option for combating bacterial infections because they are non-toxic and readily available. Various studies have investigated their antimicrobial and inhibitory effects against both Gram-negative and Gram-positive bacteria, as well as yeasts (Cai et al., 2018; Hayat et al., 2018). In addition to MgO-NPs, magnesium fluoride NPs (MgF2-NPs) have also been explored as a means of inhibiting biofilm formation through surface modification and have shown potential as antibacterial agents in several studies (Lellouche et al., 2009, 2012; Tamilvanan et al., 2008).

Iron Oxide Nanoparticles (IO-NPs)

Iron oxide nanoparticles (IO-NPs) have unique magnetic properties, high biocompatibility, and a large surface-to-volume ratio that make them well-suited for various bioprocess applications (Ebrahimi et al., n.d.; Ebrahiminezhad et al., 2016). Studies have shown that IO-NPs are effective in reducing biofilm growth on implant surfaces, which is a common cause of implant failure (Thukkaram et al., 2014). Furthermore, research has demonstrated that IO-NPs coated with 3-aminopropyltriethoxy silane (IO-NPs3-APTES), in contrast to naked IO-nanoparticles (i.e., superparamagnetic iron oxide (SIONPs)) can effectively disrupt stubborn biofilms.

Additionally, both, SIONPs, as well as IO-NPs, have been found to exhibit antimicrobial/biofilm properties against bacteria such as P. aeruginosa and S. aureus (Akbari & Ali, 2017; Sathyanarayanan et al., 2013).

In additional, Numerous studies have highlighted the potential of various metal and metal oxide nanoparticles for disrupting or inhibiting microbial biofilms. For instance, Shakibaie et al. synthesized selenium nanoparticles (Se-NPs) and tested

their effectiveness against biofilms caused by multiple strains of P. aeruginosa, S. aureus, and P. mirabilis (Shakibaie et al., 2015). Copper nanoparticles (Cu-NPs), nanofibers containing copper, and tungsten (W) and molybdenum (Mo) nanoparticles dispersed in alkyl alkoxysilane polymer have also demonstrated antibiofilm effects (Ahire et al., 2016; Chari et al., 2017; Ghasemian et al., 2015; Ogawa et al., 2017). Additionally, Chrzanowska et al. have identified the relevant nanoparticles, namely, zirconium oxide, as well as aluminum oxide to have activities against biofilm (Chrzanowska & Załęska-Radziwiłł, 2014). Moreover, some NPs (e.g., titanium dioxide and calcium fluoride) have shown capability to reduce the formation of biofilms and hence, may be useful in different industrial set-ups (Kulshrestha et al., 2016; Maurer-Jones et al., 2013).

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Chapter 10
Probiotics and Delivery System

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Probiotics

Introduction and History

Probiotics are microorganisms found in the gut microbiota, which are essential in promoting the health effects of the host through the prevention of diseases. Probiotics are defined as organisms that are essential, especially when used in required quantities, to the host owing to various health benefits. The history of probiotics traces back many centuries. In the early 1900s, Luis Pasteur became the first researcher to identify some of the microorganisms deemed responsible for initiating the fermentation process (Gasbarini et al., 2016).

Probiotics originated from the Latin word "pro," which means life. This term was first coined in 1953 by Werner Kollath, a German scientist, to refer to the active substances which were important in prompting healthy life and development (McFarland, 2015). In 1965, the probiotic term was used by Lilly and Stillwell, to refer to substances that were secreted by organisms to enable the development of other microorganisms. It was, however, until 1992 that Fuller defined the term 'probiotics'by referring them to the live microbial feed supplements that benefit the host by improving its intestinal microbial balance (McFarland, 2015).

The modern history of probiotics began in the 1900s when scientists like Pasteur discovered and identified microorganism using the pioneering studies of E. Metchnikoff, who worked alongside Pasteur to identify some of the organisms that were effective in initiating fermentation. Metchnikoff was keen on understanding microbes' main possible practical effect on human health. This forced him to

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study the Bulgarian bacillus, which was present in the Bulgarian fermented dairy products, asserting the role of *Lactobacillus* on the longer lives of the Bulgarians rural people who primarily consumed fermented foods (Gasbarrini et al., 2016). According to Metchnikoff, the presence of *Lactobacilli* bacteria effectively counteracts the putrefactive effects of gastrointestinal (GI) metabolism, which is the leading cause of aging and illnesses. Hippocrates concurred with this assertion and declared that death sat on the bowels 2000 years ago (Gasbarrini et al., 2016). Metchnikoff identified *Lactobacilli* as a probiotic because of their role in preventing aging and also influencing health. Food fermentation began long ago – The neolithic period, during the Stone Age when man started domesticating animals. The main reason for the change in how food was stored and consumed was the result of the serendipitous contamination of food, as proven by the fourth-century Chinese handbook on severe diarrhea and food poisoning. Metchnikoff defined these bacteria as the 'putrefying bacteria', which are today known as the *proteolytic clostridia*. According to Metchnikoff, adapting the intestinal microbes to food makes it easier for them to modify the flora in the human body, thus replacing the harmful microorganism, which explains the role of probiotics (Gasbarrini et al., 2016). Metchnikoff's findings were crucial in the creation of the first dairy firm in France, which used bacillus bulgaricus in the fermentation of milk. In recent years, the development of new technologies through the selected strains has influenced the production of dairy products such as yogurt, which are classified as functional foods.

Nonetheless, the history of probiotics dates back to human history because it is related to the application and consumption of fermented foods. Fermentation started immediately after a man began farming, estimated to be over 10,000 years ago (McFarland, 2015). The increased population and nutrition resulted in men looking for an alternative way of storing food and making traditional beverages. The Sumerians are the first people to practice farming and animal husbandry. In the Stone Age, milk consumption in ancient India was essential in promoting healthy and long life. However, the initial evidence of the milking practice emerged during the excavation process in Ur city, where a man is seen sitting on a stool and squeezing the cow nipples to produce milk in a bucket. Fermentation of milk is traced to the ancient people of eastern cultures, Phoenicians and the Egyptians, whereby milk was stored in bottles that were made from the skin or the stomach of the animals where the milk was collected from as a way of allowing the bacteria to come into contact with the milk and spur the process of fermentation. Milk was sometimes left in the hot environment, especially in the Turkish desert, where it turned into the thick cream, today is known as yogurt (McFarland, 2015). This aspect thus explains that despite the complexity of tracing the origin of probiotics, their healthy befits have been known since immemorial. The consumption of yogurt has been the typical food among the Turkish because they believe it provides inner well-being and a prolonged lifespan. In the sixteenth century, Suleiman discovered that yogurt effectively treated severe diarrhea.

By the seventeenth century, some regions of Italy believed that specific beverages were essential in preventing intestinal infections; thus, explaining the advent of the fecal bacteria therapy whereby the bolus of the suspended feces was obtained

and then directly infused into the patient's colon. This therapy was first reported by a group of Colorado surgeons who successfully treated four patients suffering from pseudomembranous enterocolitis in 1950 (Cammarota et al., 2015). Fecal microbiota transplant gained popularity due to its efficiency, especially with individuals suffering from *Clostridium difficile* infections. The New England Journal published the first results of the randomized trials on fecal transplants in 2013, which compared the therapy on patients with vancomycin. However, the problem ended earlier than expected because only about one-third of the vancomycin patients recovered, while those under fecal transplant recovered at a more significant percentage (94%) with just a single treatment (GS & AJ, 1958). This history explains the intestinal microbiota of probiotics and their health benefits which are popular today in treating various infections in the body.

Probiotics have a wide range of health benefits, and to achieve their desired effects on health their viability should be over 10^6 CFU/g. On the other hand, probiotics are extremely sensitive to environmental factors such as temperature, moisture, oxygen, and pH. In addition, when the probiotics are ingested, they face a high-acid environment caused by gastric juice which adds more challenge for probiotics to play their roles in the human gut. Therefore, many systems and strategies have been developed for the delivery of probiotics to the gastrointestinal system such as particles, emulsions, beads, hybrid, nanofibers, microcapsules, and hydrogels. The aim of this chapter is to present a brief overview of the classification, genomics, and health benefits of probiotics bacteria and current formulation techniques used to deliver the probiotics into the gut.

Classification of Probiotics

Classification of probiotics is a complex process because it involves various strains. However, the recognized classification is that probiotic products are classified based on the single and multi-strains of probiotics. Under the single stains, the species groups are classified based on the probiotic genus. Georgieva et al. (2014) assert that the taxonomic classification of probiotic lactic acid bacteria (LAB), was created based on the physiological, biochemical, and morphological characteristics of the genomic and molecular-based phenotypes. The seven core genera of microbial organisms most often used in probiotic products are *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus, Escherichia,* and *Bacillus*. Three common genera of probiotics have been extensively studied and classified, including *Enterococcus, Bifidobacterium*, and *Lactobacillus*. The genus *Lactobacillus* comprises various important probiotic species, which include *L. acidophilus* group, *L. casei* group, and *L. reuteri/L.* fermentum group. *Bifidobacterium* spp. *(B. animalis) strains*, which in recent days have been reported as some of the essential starters used in industrial production (Georgieva et al., 2014). From the genus enterococcus, the commonly investigated probiotic species include the *Ec. Faecium* strains to assert the *vanA*-mediated resistance of probiotics against glycopeptides (Fig. 10.1, Table 10.1).

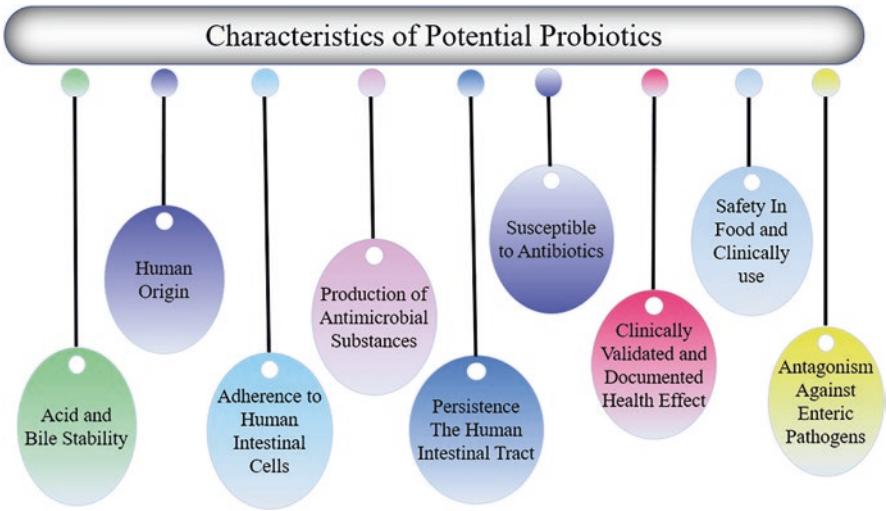

Fig. 10.1 The properties of an ideal probiotic bacteria

Table 10.1 Common probiotics microorganisms

Lactobacillus spp.	*Bifidobacterium* spp.	*Bacillus* spp.	*Streptococcus* spp.	*Enterococcus* spp.	*Saccharomyces* spp.
Acidophilus *Plantarum* *Rhamnosus* *Paracasei* *Fermentum* *Reuteri* *Johnsonii* *Brevis* *Casei* *Lactis* *Bulgaricus* *Delbrueckii* *Gasser*	*Breve* *Infantis* *Longum* *Bifidum* *Thermophilum* *Adolescentis* *Animalis* *Lactis*	*Coagulans*	*Thermophilus*	*Faecium*	*Cerevisiae*

Genomics of Probiotic Bacteria

Lactobacillus spp.

Lactobacillus spp. is classified to represent a range of LAB-denominated bacteria, which are characterized by adding the fermentation to sugar to lactic acid and other compounds. Lactobacillus are gram-positive, non-sporulation cocci and rods that grow through anaerobic processes (Campos & Mena, 2012). They are the essential bacteria that aides in producing lactic acid during the fermentation of animal-based products and vegetables. The *Lactobacillus* genus contains various bacteria and is

marketed as probiotics because of their health benefits to humans. According to Vinderola et al. (2019), the genomics of any microorganism is allocated to the environment and its size. The genomics of the *lactobacillus* species and its reduction has been associated with their adaptation in the gastrointestinal tract of humans and animals. This is because some *lactobacillus* species often acquire selective association with the host, thereby losing a larger part of their genomes via the co-evolutionary relation with the host.

An ideal example is the *L. jenseni, L. iners, L. gasseri, and L. crispatus* bacteria species of *Lactobacillus*, found in the virginal tract (Vinderola et al., 2019). These species have reduced genomes as a result of their niche specialization. Others, like *L. casei and L. Plantarum,* can be found in several environments because of their evolutionary properties and larger genomes in various niche environments. Due to the increased importance and significance of *Lactobacillus* species, especially regarding health and economic importance, there has been increased interest in modifying these species.

Kant et al. (2011) revealed that in a category of 20 *lactobacillus* ssp., there is a shared orthologous gene known as the lactobacillus core genome (LCG). A closer inspection of LCG showed that about 100 genes of *Lactobacillus* appeared like operon clusters revealing the shred functionality, control, and organization of the *Lactobacillus* species. A similar organization revealed the ancestry link and association between the management of nitrogen and sugar metabolism, which is usually conserved in all *Lactobacilli* family specie. The *lactobacillus* genome is divided into three main clusters: the NCFM, WCFS, and the GG, which are said to consist of the 8,7, and 5 genomes respectively (Kant et al., 2011). NCFM is considered the largest genome widely detected in various environments due to its evolutionary properties and is the most coherent in the *Lactobacillus* species. The NCFM is present in the genome of *L. delbrueckii, L. johnsonii, L. gasseri, L. crispatus, L. helveticus, and L. acidophilus* species. On the other hand, the WCFS, which contains the outgroup genome, is often present in species such as *L. salivarius, L. reuteri, L. fermentum, L. brevis, and L. Plantarum* (Kant et al., 2011). The GG group comprises *L.sakei, L.casei, and L. rhamnosus,* and other 30 orphan genes in which, some of which encode proteins. Such modifications make it easier to understand and analyze the genomics of *Lactobacillus* spp. The mutants effectively understand the probiotic mechanism of action exhibited by specific strains of *Lactobacillus*.

Bifidobacterium spp.

The ecological importance of *Bifidobacterium* spp. was first discovered from the stool samples of breastfed infants, which, however, declines with growth. A *Bifidobacterium* is a group of gram-positive bacteria, part of the larger actinobacteria phylum. Its ecological importance has been identified in 47 different taxa, specifically in the social gut of insects, birds, and mammals, whose offspring usually depend on parental care to grow and evolve (Milani et al., 2016). This feature seems to be the distinguishing characteristic from other gut genes, such as *Lactobacillus*

and Bacteroides, following its vertical transmission from the parent to the offspring, thus explaining its genomic features.

According to Vinderola et al. (2019), the initially fully decoded genome of *Bifidobacteria* was discovered in the human gut commensal, known as the *Bifidobacterium* longum susbsp.longum NCC2705. After this, several *Bifidobacterial* strains found sequenced genomes, especially in the adult fecal isolate known as the *Bifidobacterium longum* subsp. *longum* DJO10, and *Bifidobacterium bifidum* PRL2010 isolates in the infant fecal. Others include *Bifidobacterium longum* subsp. *infantis* ATCC 15697 and *Bifidobacterium breve* UCC2003. Also, fully decoded genomes of *Bifidobacterium* have been discovered in the oral cavity of humans, which include the isolate of *Bifidobacterium dentium* Bd1 and several strains of B*ifidobacterium animalis* subsp. *Lactis* taxa (Milani et al., 2016). These two categories have, in recent years, attracted increased attention and interest due to their perceived health benefits to both animals and humans.

By 2014, only about 10 of the 47 genome sequences of *Bifidobacterium* were available and fully decoded. However, since then, several specifies of the *Bifidobacterium* genus have been recognized to belong to a specific genus that is gnomically decoded, thus representing the genomic encyclopedia for exploring the genetic variability of this genus. After the reclassification of *Bifidobacterium stercoris,* which means *Bifidobacterium adolescentis,* the current taxon of *Bifidobacteria* remains at 47 (Milani et al., 2016). Characterization of all species of the *Bifidobacterium* genus showed that the gnomic size of its species ranged between 1.73 to 3.25 Mb. Some species, such as *Bifidobacterium indicum, exhibit a* size of 1.73, which is the minimum, and others, like *Bifidobacterium* biavatii, depicted a genomic size of 3.25 Mb, which is the maximum (Milani et al., 2016). This size range effectively corresponds to the protein-encoding predictions of 1352 and 2557 of the reading frames. This range explains the remncien evolutionary pathway in many *Bifidobacterium* species that they use in the genome loss or acquisition while associating with their hosts.

The functional classification of the entire *Bifidobacterium* taxon showed that about 13.7 of the identified genes encode scenic enzymes, which are effective carbohydrate metabolism higher than the commensals. It is also evident that a more significant percentage of these genes are applied in the glycan, revealing a notable presence of the 47 *Bifidobacterial* species in the core genome (Milani et al., 2016). One important aspect to note is that the core genomic code sequencing of bifidobacterial species is that these species usually encode through the utilization of the bifid shunt enzymes. It is presumed that successful evolutionary pathways of *Bifidobacteria* result from specific metabolic pathways that prompt the creation of ATP compared to other ways, such as glycolysis. Also, *Bifidobacterial* success is attributed to the unique metabolism using glycan or dietary pathways. Milani et al. (2016) assert that the identification of the “truly unique genes” (TUGs) accounts for about 14–64% of the *Bifidobacterial* pan-genomes that consist of the proteins utilized during carbohydrate metabolism.

Saccharomyces spp.

Over the years, yeast has been used in the fermentation of wine and beer. Due to continuous development, starter cultures are prominent in commercial and industrial production today. *Saccharomyces* yeast genus have, over the years, been widely used in food fermentation due to their ability to stand stressful conditions (Giannakou et al., 2020). Several studies on the genomics of saccharomyces strains show differing characteristics in the recombination patterns and rates even among the related and connected genus through their difference in characteristics (Giannakou et al., 2020). Development and advancement in sequencing technology have been essential in identifying the genetic composition of various yeast strains through differing phenotypes determined by the plethora of environments. A recent study by Giannakou et al. (2020) shows that the current large-scale phenotyping and genome sequencing of the *S. cerevisiae* industrial strains used in brewing are phenotypically and genetically isolated from their initial ancestors when exposed to artificial conditions. This investigation shows that the brewing yeast used in industrial production is tolerant to stressful conditions resulting in the deletion and insertion of small and large fragments discovered in various trains.

The genomic rearrangement of saccharomyces and the use of copy number variations positively contribute to altering the expression network of the genes. Any form of improvement in the genomic rearrangement results from the genomic and environmental changes and the changes in the transcriptome. Giannakou et al. (2020) debates that differences observed in the copy number variations result from the genes utilized to metabolize maltotriose and maltose. The copy number variations are considered an essential adaptation means for the saccharomyces genes to adapt to the changing environmental conditions. The maltose metabolism process comprises three gene groups, MALR, MALS, and MALT, which consist of the regulator proteins, maltase, and maltose transporters, respectively. Differences in copy numbers and chromosomal locations of the utilized MAL genes are usually observed in the commercially used strains (Giannakou et al., 2020). For example, about 15 MAL copies of the German yeast strains were discovered in the MAL31 gene. The breakdown and uptake of maltose, which acts as the principal carbon source in beer fermentation, is significant in supporting the performance and survival of the yeast strains.

The *saccharomyces* genome of the larger brewer's yeast is a hybrid with *Saccharomyces cerevisiae and Saccharomyces eubayanus, t*ermed the sub-genomes. This is due to their specific application in the industrial brewing of beer for commercial use. A study by Giannakou et al. (2020) showed that the genome size of the saccharomyces of 22.7 Mbp consists of 133 scaffolds and 65 scaffolds whose size exceeds 10 kbp. Concerning the annotation sequence provided by Giannakou et al. (2020), about 9939 genes were submitted. Approximately 53.93% responded to the *S. cerevisiae* genes, while others comprised those of the *S.eubayanus* (Fig. 10.2).

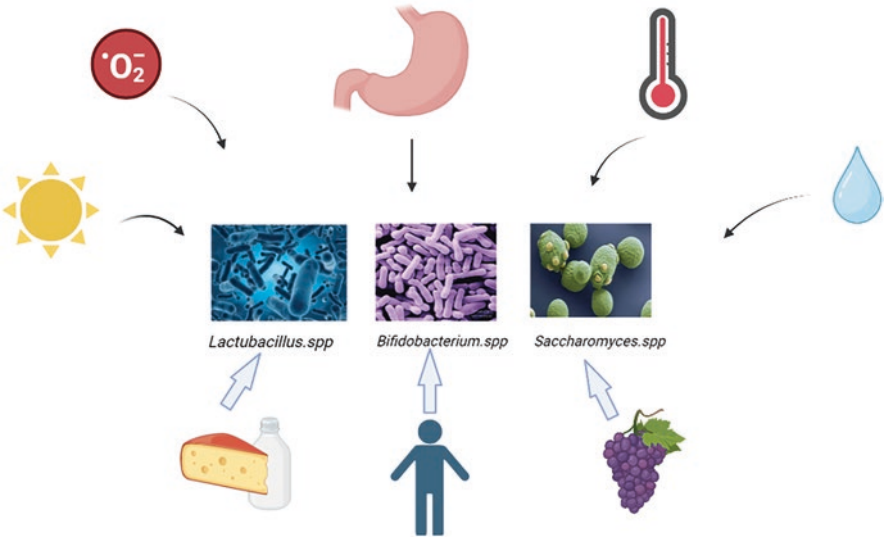

Fig. 10.2 The origins of three common probiotics and environmental factors

Probiotics and Human Health

Probiotics in Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a clinical disorder comprising of ulcerative colitis and Crohn's disease because of their striking similarities. Although the etiology of IBD remains unknown, it is evident that it is caused by various environmental, microbial, and genetic factors. The complexity of the IBD disease patterns dictates that there is a need to conduct extensive research on applying probiotics to treat and manage IBD-related diseases based on specific strains. Preidis et al. (2020) notes that probiotics play a crucial role in intestinal microflora, which supports the development of severe gut inflammation. One of the main bacterium strains that contribute to chronic gut inflammation in mice. Studies have, however, revealed that the application of particular probiotic bacteria is essential in the minimization and prevention of intestinal inflammation.

A study conducted by Kneifel and Salmine (2010) showed that administration of probiotics has been successful in the treatment of IBD in humans. Probiotic bacteria have been proven effective in counteracting the inflammatory process that contributes to IBD through enhanced degradation of neural antigens. Probiotic bacteria also reduce the secretion of the inflammatory mediators, thereby improving the standardization and normalizing the indigenous flora, which positively contributes to stabilizing gut affect barrier functions (Kneifel & Salmine, 2010). One of the most significant rationales in probiotic therapy in IBD is the restoration of the indigenous microflora properties of the particular strains of probiotic bacteria. Some of the best-documented strains of probiotic bacteria used in the treatment of IBD

include the VSL #3mixture, which is comprised of eight strains that consist of streptococcus, bifidobacteria, and lactobacilli.

Another proven health benefit of probiotic bacteria in IBD is in treating pouchitis. Preidis et al. (2020) asserts that about 50% of individuals who often undergo ulcerative colitis surgery usually develop pouchitis. This condition usually results in urgent and frequent bowel movements, fever, bleeding, and abdominal cramping. One of the known treatments used by many humans suffering from pouchitis is the use of antibiotics. However, the use of antibiotics is not practical because of the recurrent inflammation, which recurs in about two-thirds of the patients suffering from pouchitis. The leading cause of pouchitis is unknown. However, the condition has been attributed to the reduced presence of some essential bacteria in the intestinal tract. A study conducted by Preidis et al. (2020) on the effect of probiotics among patients suffering from pouchitis showed that after 9 months of treatment, 85% of the group under the probiotic bacteria were symptom-free, while 100% of those under the placebo group experienced a relapse in 4 months. This shows the effectiveness of the probiotic bacteria in treating and managing pouchitis compared to the use of antibiotics, which shows recurring effects of inflammation.

Probiotics and Pediatric Diarrheal Disorder

Pediatric diarrheal disorder in children is one of the disorders defined by the World Health Organization (WHO) as an illness that is presumed or proven infectious etiology, lasting less than 14 days. Diarrhea in children, especially in developing countries, has been the major cause of higher morbidity and mortality rates among children under 5 years (Vandenplas et al., 2013). Although there are several causes of pediatric diarrheas, it has been associated with bacterial, virus, and parasite pathogens, which contribute to acute diarrhea, which in worse cases, is associated with persistent diarrhea that lasts for more than 14 days and contributes to higher hospitalization and even death. The management of pediatric diarrhea is difficult because of its complex pathogens and etiology. There are several management mechanisms applied, which include the use of antimicrobials, rehydration, micronutrient supplementation, and also through enough dietary management. In many developing nations, the pediatric diarrheal disorder is a common problem determined by the frequent recurrence of diarrheal episodes due to nutritional compromise. Some common risk factors for diarrhea in children below the age of five include immune deficiencies and breastfeeding.

Various studies have shown the positive effects of the use of probiotics in managing diarrhea among pediatrics due to the health benefits it accrues to the host (Vandenplas et al., 2013). Probiotics are widely used in managing pediatric diarrheas because of the lack of adverse effects and widespread acceptance. Some of the commonly used probiotics include Lactobacillus, Streptococcus, and Bifidobacterium. Several studies have demonstrated that probiotics contain a safety profile by reducing the duration of diarrhea in children between 13.4 and about 30.5 h (Vandenplas et al., 2013). Other effects have been reported in reducing stool

frequency, which contributes to the reduction in hospital stay duration. The rationale behind the treatment of pediatric diarrhea through probiotics is the assumption that probiotics can modify intestinal microflora composition. The probiotic also has the potential to wear out the enteric pathogens that contribute to diarrhea in children.

The effectiveness of probiotics in managing diarrhea is based on three main mechanisms. The first mechanism is through the luminal mechanism, where they inhibit the growth of non-homologous strains through the production of hydrogen peroxide, lactic acid, and fatty acids that lowers the intraluminal pH creating hostile conditions for the development of diarrhea-related pathogens (Vandenplas et al., 2013). Some probiotics, such as saccharomyces boulardii, have shown their potential in reducing toxins from pathogens such as Escherichia coli, vibrio cholera, and clostridium deficile. Another means is mucosal through probiotic agents that upregulate the production of protective trefoil and mucins. Lastly, probiotics effectively manage diarrhea from submucosal by strengthening the innate immune system.

Anticarcinogenic Effects of Probiotics

Dos Reis et al. (2017) showed the relationship and association between using an enriched diet with *Lactobacillus* and the possible decline in colorectal cancer. The results of this study showed some of the important features of probiotics in the modulation of apoptosis and proliferation of cancer cells, such as myeloid leukemia and colonic and gastric cells. Probiotics have been proven to contain anticarcinogenic properties, effectively reducing human cancer-related tumors. Some probiotics, such as *L. rhamnosu and L. plantarum,* have proved to be effective in creating all-induced cancer in the rat experiment Dos Reis et al. (2017). However, these results show that the interpretation needs to be taken with caution because the tumors were indicated through the initiation of various chemical agents, usually different from the natural carcinogenesis process. Śliżewska et al. (2020) asserted that the probiotic effects and antiproliferative role of some probiotic strains against carcinoma cells show that the regimens of probiotics can be used in preventing cancer development and during chemotherapy treatment. For example, Śliżewska et al. (2020) showed that some of the probiotics that are beneficial to gastric cancer treatment include *Lactobacillus reuteri* PTCC 1655 and *Lactobacillus kefiri P-IF.* Colorectal cancer is treatable and manageable by probiotics such as *Bifidobacterium longum* BL-88, *Lactobacillus acidophilus* LA-11*, Lactobacillus acidophilus, Enterococcus faecalis, and Bifidobacterium lactis Bb12* among others.

The anticarcinogenic effects of probiotics take place on various mechanisms, which include modifying the intestinal microbiota, metabolism in the microbiota, improving the intestinal barrier, and influencing the carcinogenic and other mutagenic factors that prevent the development of cancer (Slizewska et al., 2020). Other mechanisms include the production of conjugated linoleic and short-chain fatty acids and inducing the apoptosis and inhibition of the cancer proliferation cells.

Potential Pharmaceutical Applications of Probiotics

As aforementioned, probiotics contain several health benefits for humans and animals. Among the common forms of administration of probiotics is through oral delivery, which has, in recent years, proved to be one of the most patient-compliance forms of administration. Oral delivery of probiotics is administered in three primary forms, which include hydrogels, oral films, capsules, and tablets. Also, the microencapsulation and surface coating technologies in the formation of probiotics, whether used as dietary supplements or drugs, have contributed to the stability of probiotics in the GI tract (Iravani et al., 2014). One of the commonly used protein polymers in microencapsulation include synthetic polymers, lipids, polysaccharides, and other protein-based polymers, which are essential in increasing the ability of the probiotics to resist harsh environments. These polymers protect the probiotics from gases and moisture by forming a thin film.

Nasal delivery is another potential pharmaceutical application of probiotics that has proved effective in managing airway-related diseases. Dysbiosis, which refers to the imbalance of the microbiota, is the primary cause of inflammatory illnesses such as asthma and chronic rhinosinusitis. Mercenier et al. (2003) assert that probiotics can alleviate dysbiosis by interacting with the recognition receptors that signify the microbe's molecular composition. Additionally, probiotics delivered through the nasal restore the epithelial barrier through adherence interactions and junctions. Nasal delivery of probiotics is easy to administer, non-invasive, and also avoids the harsh and acidic environment of the gastrointestinal tract.

The tropical skin delivery of probiotics is an application of probiotics to the skin, which has proven a practical approach in treating various illnesses. This is because the skin microbes in the dermis and the epidermis in the maintenance of the health of the skin and also in preventing the entry of pathogens, thereby generally regulating the skin's immunity (Kaur et al., 2002). A significant percentage of probiotics administered through the skin procures higher amounts of lactic acid, thus preventing the growth of pathogenic bacteria. These probiotics are administered through microneedles, sonophoresis, electroporation, iontophoresis, and absorption enhancers.

Other probiotics administered in the virginal are essential in restoring the standard and balanced microbiota of the virginal, thus preventing infections. Healthy and normal virginal microflora consist of lactobacilli which act as a bacterial barrier against infection-causing pathogens (Baral et al., 2021). However, several contributing factors, such as hormonal imbalance, use of antifungal drugs, antibiotics, pregnancy, and menopause, contribute to an imbalance of the virginal pH resulting in vaginal infections such as vaginitis. As a result, supplements such as exogenous lactobacilli are significant in maintaining a healthy vagina through the optimization of the virginal microbiota. These can include in-situ gels, tablets, suppositories, and verdenelli.

Probiotic Delivery System

Factors Affect the Viability of Probiotics

Thermal Stress

Temperature regulation is an important factor that determines the survivability and viability of probiotics, especially during fermentation. The optimum temperatures for the growth of a majority of LAB are between 30 and 43 °C. This temperature range supports beneficial bacteria's growth and development besides enhancing their survivability in the microbiota gut. Some bacteria, such as *L. acidophilus,* used as the starter cultures for the fermentation of milk, can survive in temperatures of up to 45 °C (Wendel, 2021). However, such high temperatures, especially during processing, affect the viability of probiotics. Those bacteria found in animals, such as *B. animalis* subsp. *Lactis*, are viable in temperatures ranging from 41 to 43 °C. Any temperature above or below this range reduces the viability of the probiotic. For example, in spray-drying of probiotics, heat is one of the main stressors that affect the cellular components of probiotics, thus resulting in thermal stress, which in return leads to destabilization of the cell membranes and denaturation of proteins. Ribosomal damage is an extreme case of heat inactivation of probiotics.

Oxidative Stress

Aging is one of the most irreversible processes in all living organisms. Aging is characterized by several features, including the reduction of beneficial bacteria in the human body. As people age and the beneficial bacteria are reduced, the oxidative free radicals play an imperative role in damaging the lipids, proteins, DNA, and other important molecules, thus leading to diseases and loss of physiological functions. These oxidative free radicals are the main reason for the aging of internal organs. Some probiotics, such as *bifidobacteria* and lactobacilli, contain strong antioxidant properties that effectively reduce aging. However, as aging nears, their efficacy declines, resulting in oxidative stress and contributes to a shift in the gut microbiota due to the diminished growth of bacteria. This oxidative stress contributes to weight loss, nutrient malabsorption, and diarrhea, which are common among older adults.

Osmotic Shock

Osmotic stress is caused by the methods of encapsulation of probiotics which also affects their viability. For example, drying is one of the convenient and most preferred means of probiotic encapsulation, which include spray drying and freeze drying. Drying helps in putting the cell in a resting state for some time before

consumption. Drying involves dehydration of water which, however, increased loss on the cell membrane, which may contribute to its death. Dehydration increases the ratio between the cell volume and the cell surface, thus contributing to the deformation of the membrane (Wendel, 2021). Freeze drying, whereby the initial extracellular ice crystals are formed, contributes to an increased concentration of the medium solutes. This concentration leads to osmotic stress. The ice crystals' formation contributes to the organelles' disruption and membrane destruction, thus negatively affecting their viability.

Gastric Juice

Gastric juice is one factor that affects the viability of probiotics. Probiotics are faced with the challenge of surviving in the stomach, producing gastric juice, thus exposing the LAB bacteria to higher concentrations of pepsin and low pH (Govender et al., 2014). Gastric juice provides unfavorable and harsh conditions for the survival of probiotics because these conditions may cause cell membranes' inactivation and eventual death. However, to survive in these harsh conditions, recent technology in the formation of probiotics to either capsules or powder-form products improves their viability in the GIT.

Delivery of Probiotics

Encapsulation of Probiotics

Microencapsulation refers to the mechanical or physiochemical process allowing the entrapping of the bacterial class with coating using various hydrocolloidal materials. These coatings offer enhanced probiotic protection from harsh conditions when exposed to various environments, including antimicrobial agents, heat shocks, molecular oxygen, cold shock, bile salts, low pH levels, and high acidity (Iravani et al., 2014). The main goal of creating this protective layer is to reduce cell loss and injury, thus increasing the viability of probiotics. The commonly used material for encapsulating probiotics comprises gastro-resistant material, which is applied to amplify and accelerate the benefits and health effects of probiotics in the human body. Microencapsulation is a concept that is extensively applied in the pharmaceutical industrial production of various drugs and supplements. Yadav et al. (2013) define microencapsulation as a process by which small droplets and particles produce microcapsules in separating the core function materials from adverse conditions. On the other hand, encapsulation is the method through which a coating is created across the inner cell matrix of the capsule wall. The main role of encapsulation is to enhance the stability of the cells during production, which in return would improve their viability during storage. There are several approaches used in the encapsulation of probiotics. These methods are classified into two broad categories: silkworm cocoons and spider webs (Fig. 10.3).

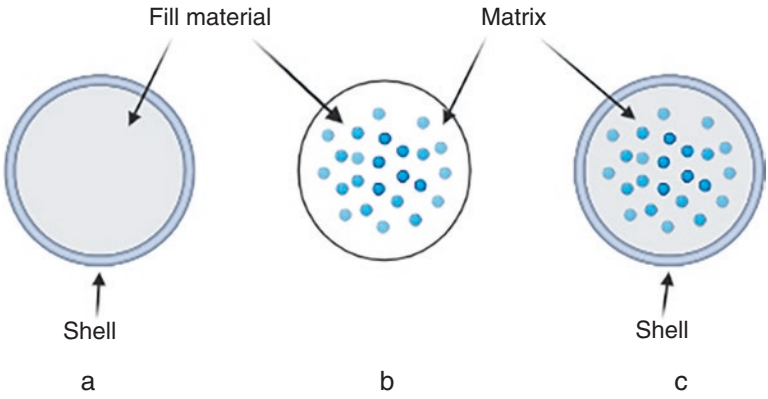

Fig. 10.3 Schematic representation of encapsulation systems: (**a**) reservoir type, (**b**) matrix type, and (**c**) coated matrix type

Some Techniques Used for Encapsulation of Probiotics

Spray Drying

Spray drying is a method that is used in the industrial microencapsulation of probiotics. This method involves the dissolution of a polymer when exposed to the continuous stage where the primary particles are surrounded by sprayed droplets. The spray-drying process shrinks the cell to a real polymer that encloses the probiotic material (Iravani et al., 2014). The process has been proven to be one of the cost-effective approaches that can be applied in producing a large quantity of probiotic cultures. However, this is only possible in crucial determinants such as controlled outlet air temperatures, air pressure, and the atomization category applied in the process. Spray drying is the standard approach used in producing microcapsules using differing nozzle temperatures and polysaccharide mixture, all of which enhance probiotics'survivability and viability.

The spray drying method is one of the common techniques used in the large-scale manufacture of microcapsules. It is also arguably the best approach in the development of dry microcapsules formed from the water-insoluble through the use of controlled and small particles. Spray-drying is a desirable system usually incorporated in the production and growth of effective probiotic bacteria used in different foods. Various factors that make it effective include the composition of the organoleptic characteristic of food, sensory properties, limited effects, easier storage and handling of the starter cultures, and higher stability during production and storage (Iravani et al., 2014). However, this method rarely uses microencapsulation to offer a protective layer to the active material and the cell membrane because it contributes to cell damage, which may cause death due to inactivation and continuous dehydration. Exposing the probiotics to higher temperatures during spray drying negatively affects the viability of the products. Expiry due to higher temperatures also reduces

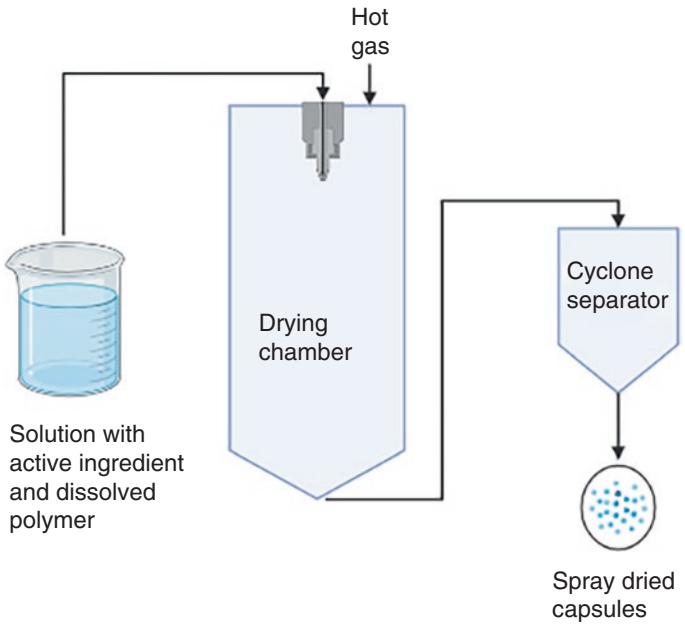

Fig. 10.4 The encapsulation process of probiotics by spray drying

the efficiency and biological function of the end product, thus reducing its ability to serve other functions as stipulated. Also, during spray drying, water is dehydrated from the product. This process of water removal from probiotics to form tablet or powder-form products may result in irreversible effects on the functionality and structural integrity of the protein and cell membrane of the bacteria. One of the ways to prevent and reduce these drawbacks includes using milk fat droplet coats containing powdered particles. These particles are then freeze-dried through the emulsification process under the whey protein polymer and spray-dried using the continuous two-step process (Fig. 10.4).

Freeze-Drying

Freeze drying, on the other hand, frees the product below the critical temperatures used in its formation. Freeze-dying is a technique that has the potential to be used in the industrial production of probiotics and enhance their viability period, especially the storage period before they are consumed. After the product has been frozen below its normal formation temperature, it is dried in a chamber where water from the product is removed through sublimation, increased shelf temperatures, and reduced pressure levels (Iravani et al., 2014). After the primary drying chamber, the products are transferred to the secondary drying stage, where the available water is removed through desorption, and the product is slowly transformed to ambient

conditions. Some of the most significant factors when undertaking the freeze-drying processes include the medium of the pH levels, temperature, the freezing rate, the initial cell concentration, prehistoric biomass, and the composition of the protective compounds. All these conditions must be effectively contained and managed to produce quality end products with a longer viability period. Osmotic shock is the main contributing factor that affects the viability of probiotics resulting in cell membrane injury through recrystallization and intracellular ice formation.

Spray-Freeze-Drying

The spray-freeze-drying (SFD) is a concept of encapsulation that traces back to the 1940s when the process was applied in the successful production of protein particles through varying surface areas (Vishali et al., 2019). SFD process involves spray freezing the mixture through the liquid nitrogen bath, and then the solution is vacuum-freeze dried. SFD takes advantage of the key features of spray drying and largely comprises the atomization of a liquid to produce smaller and fine particles, which are achieved through freeze drying (Vishali et al., 2019). This contains a certain value when the sensitive materials are thermally dried to produce powders in an enhanced, stable, and controlled way. SFD has, in recent years, been used as the standard method for the production of dosage drugs used in the pulmonary application and needle-free injections, inhalable dry powder drugs, and preparation of BCS class II formulation drugs which are essential in enhancing their dissolution characteristics, as well as other powders that have specific functions. Like other encapsulation methods, SFD also contains some limitations, including its fast cooling, which enhances the development of large ice-glass interfaces and easily causes the denaturation of proteins.

The SFD undergoes a three-stage process. The feed solution is spritzed with an atomizer. The atomized particles are then frozen by exposure to low and medium temperatures that lock them to the spherical shape. The frozen spherical droplets are then transferred to the freeze-drying chamber, where water is sublimed through drying to achieve dry powders. The morphological and physical properties of the produced powder are only altered during the first two steps, with the last step only used in obtaining a dry product (Vishali et al., 2019). The SFD technique is essential in encapsulating probiotics because of its increased management of the residual moisture's particle size, density, and content. Unlike other methods, SFD makes it easier for the experimenters to manipulate the encapsulation process parameters such as the choice of the atomizer, the concentration and chemical composition of the feed solution, the cryogenic liquid temperature to be used, and thus having an idea of the end product before it is produced.

Vacuum Dry

The dry vacuum procedure is a microencapsulation approach in which a vacuum dryer contains a chamber aligned with various heated shelves. In these shelves, trays that contain wet biomass are arranged on the layers to allow the evaporation of water. The water vapor from the wet biomass is dried through a vacuum pump and later cooled in the condensing chamber. During freeze drying, the cells are frozen to allow for the removal of water from the cells. In vacuum drying, the cells are, however, allowed to remain in liquid form. Also, a larger percentage of the vacuum dryers used for the microencapsulation of probiotics usually function under higher pressure and temperature levels. As a result, the energy consumption rates of vacuum dryers are 40% less than that used in spray drying and freeze-drying techniques. Ermis (2022) asserts that the optimal pressure levels for the vacuum dryers used in encapsulation are between 30 and 60 millibar, which correlates with the specific boiling point of water, which is between 25 and 30 °C. However, one of the main disadvantages of vacuum dryers for encapsulation is that it consumes a lot of time during the process, estimated to be between 20 and 100 h, depending on the type of probiotic encapsulated (Ermis, 2022). Recently, a new development in vacuum drying has been discovered, including the application of pulse-spouted microwave vacuum drying (PSMVD) used in drying banana cubes. The dried cubes portrayed an expanding trend, with a better rehydration ratio and structure and high nutritional value compared to the traditional vacuum drying method.

Extrusion Technique

The extrusion technique is considered to be the cost-efficient, simple-to-use method that reduces cell injuries, thus increasing the viability of probiotics. Two of the common characteristics of this technique is flexibility and biocompatibility. However, extrusion microencapsulation is not appropriate for commercial production because it is slow, thus contributing to a low yield of microbeads (Iravani et al.., 2014). Extrusion forms bead with larger sizes compared to those formed by the emulsion technique. Regarding the capsules, the size is determined by a range of factors, including the collecting solution of calcium chloride, the distance of the syringe, the diameter of the extruder orifice, and the viscosity of sodium alginate. Larger particles are formed through increased concentration of sodium alginate in higher viscosity. Encapsulation of probiotics is done through the extrusion technique in a plasticized matrix of a composite comprising starch, flour, and fat. Then, the paste from the mixture is chopped to obtain particles of a diameter range between 0.5 and 1.5 mm (Iravani et al.., 2014) (Fig. 10.5).

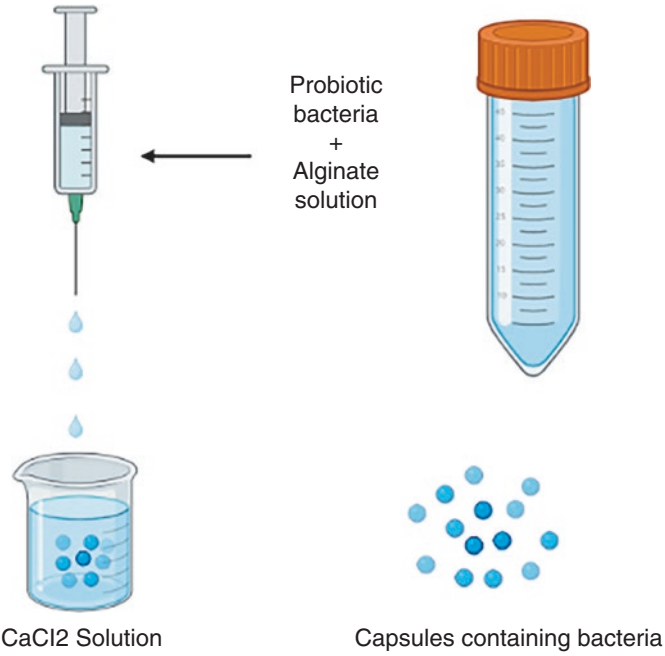

Fig. 10.5 Extrusion encapsulation method

Emulsion Technique

Unlike the extrusion method of encapsulation of probiotics, the emulsion method is more expensive and complex. This is due to the requirement for oil during the emulsion preparation, whereby a small portion of the polymer slurry or the cells, known as the dispersed stage, is added to the overall volume, termed the continuous process of the vegetable oil. Some of the commonly used oils include those extracted from light paraffin, corn, soy, and sunflower. The first method that was used in entrapping the required bacteria is the use of oil or water during encapsulation. At the beginning of the emulsion encapsulation process, the material used for encapsulation is added to the probiotic genus. The solution is dangled in an 80-tween oil bath. KCL, κ-carrageenan, and *Sodium alginate* are used as emulsion breakers and have been proven to microencapsulate probiotic bacteria under the emulsion technique (Iravani et al.., 2014). The type of emulsifier, the agitation rate of the mixture, viscosity of the encapsulated mixture before the addition of gelation are the key determinants that define the diameter of the microbeads formed at the end of the process. Larger beads of about 1000 μm are prone to having weaker coated membranes and a course structure. Iravani et al. (2014) assert that the diameter of the produced micro beads influences probiotics' sensory, metabolic, and viability properties. The diameter is also a key determinant in the absorption and distribution of some quality microbeams produced (Fig. 10.6).

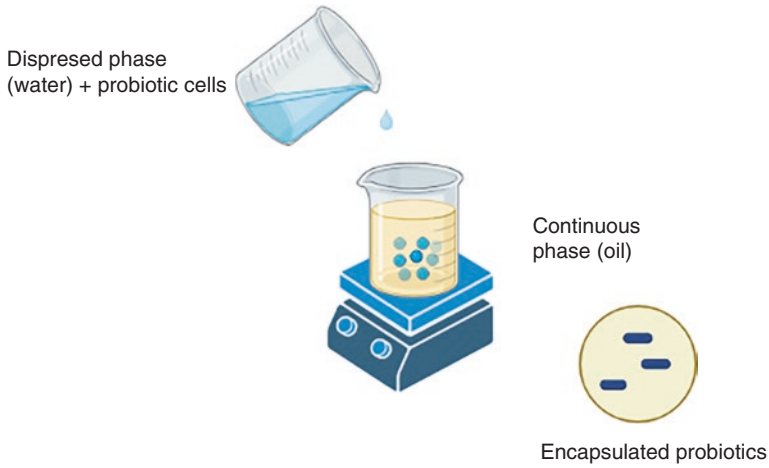

Fig. 10.6 Emulsion technique of probiotics

3D Printing

Currently, there is an increased demand for the production of functional foods due to their health benefits to the body. This increased demand has resulted in the need to deliver probiotics, which are classified among a larger percentage of functional food through various food matrices. In recent years, 3D printing technology has traced increased attention, especially its usage in the food industry (Yoha et al., 2021). This is because this encapsulation technique permits the production of customized products based on the market's needs. The 3D printing method enhances the protection of probiotics, thus prolonging their viability. The *lactobacillus Plantarum* cells are first dried via the traditional encapsulation process. Later, a *fructooligosaccharide* matrix, whey protein, and maltodextrin, which is prebiotic, are added to the base in a ratio of 4:1:1, respectively. The drier microcapsules are used in the process of 3D printing through the composite flour via CARK 3D printing. According to Yoha et al. (2021), printing the final product using the 3D method did not alter the viability of the probiotics used in the process. The effect of the probiotic stability, especially at the storage level, was similar to the four traditional methods used. The freeze-drying recorded a 90% stability and viability after the post-processing, while those manufactured through spray-freeze-dried and incorporated with freeze dying after the post-3D printing recorded a 79% best survival rate and increased viability of 6.43 ± 0.17 $\log_{10}$ CFU/ml when exposed to the static vitro digestion environment (Yoha et al. (2021). This shows that 3D printing technology is an improved technique that enhances the viability of probiotic bacteria produced by the traditional encapsulation methods, thus enabling them to be delivered in various customized shapes and sizes to serve various functions. The 3D printing technique of encapsulation contributes to the different production of probiotics to fit the current needs in the market, which the traditional microencapsulation approaches cannot perform.

Food-Grade Delivery Systems for Probiotics

Two main food-grade delivery systems are used in the protection of probiotics; including spider webs and silkworm cocoons. Just like the natural substances in insects, silkworm pupae contain similar biological features, such as those exhibited by probiotics (Gao et al., 2021). The encapsulation of probiotics has been inspired by the entrapped layer of spider-web-like networks or the silkworm cocoons, which have been proven to be the two common types of probiotic delivery systems. This section delves into the silkworm cocoons and the spider webs.

Silkworm Cocoons

Particles

Particles are part of the mature oral delivery system, classified into microparticles and nanoparticles based on their sizes. The standard size for differentiating these two classes is 100 nm.

Microparticles are large compared to nanoparticles, thus offering enough space to encapsulate probiotics. Various ways have been deployed in the preparation of microparticles used in the delivery of probiotics. The first method is electrostatic interaction and sprays chilling, which effectively fabricates microparticles to deliver *Bifidobacterium animalis subsp. lactis BLC1* in stressful and GI environments. The second method is through coacervation, which involves preparing solid lipid microparticles using the gum Arabic and the concentration of whey protein (Gao et al., 2021). These compounds have been proven effective in the encapsulation of Bifidobacterium *animalis* subsp. *Lactis*, with a viability period of up to 120 days. The last method is through internal ionic gelation and emulsification to formulate pectin microparticles, whereby lactobacillus acidophilus LA-5 is added to prolong the viability and stability of this probiotic for 120 days.

The second category, the nanoparticles, has a large surface area-to-volume ratio despite their smaller size. Nanoparticles have been proven suitable for encapsulating nutritional components as a delivery system. Ebrahimnejad et al. (2017) showed that the mixture of tripolyphosphate anions and chitosan formed the chitosan nanoparticles through ionic gelation. Chitosan nanoparticles are used to encapsulate lactobacillus acidophilus, thus increasing the viability of probiotics under the gastric and intestinal environments. However, it is important to consider the selection of nonhazardous materials in the formation of the nanoparticles because the use of zinc and silicon dioxide has proved harmful to the cell membrane of probiotics such as *Lactobacillus plantarum*. Thus, it is important to create dry particles to enhance the required lifeline of probiotics.

Emulsions

Today, various emulsions are used in delivering probiotics. These include O/W emulsions, W/O emulsions, W/O/W emulsions, and W/W emulsions. Fabrication of the O/W results in forming of a high-internal phase and the O/W nanoemulsions, which serve as the crucial system for probiotic delivery. The W/O emulsions exhibit the actual protective effects due to the embedment of probiotics into the inter-water phase (Gao et al., 2021). The use of W/O emulsion made of acrylic acid polymer in the encapsulation of lactobacillus Plantarum CIDCA 83114 has the potential of controlling probiotic delivery in the gut with the addition of *oleogels.* The addition of oleogels prolongs the survivability of *Bifidobacterium lactis* and *Lactobacillus acidophilus* up to 40 days of storage.

The water-in-water emulsions undergo a continuous dilution, and the liquid coacervates the concentrated phase. A study by Singh et al. (2018) utilized sodium carboxymethyl cellulose and B pigskin gelatin to prepare W/W emulsions with the encapsulation of *Lactobacillus rhamnosus GG LMG 18243* into the systems. Later, a heteroprotein coacervation was added with the role of entrapping *Lactobacillus reuteri TMW1.656* to the water-in-water emulsion (Gao et al., 2021). During this process, the protective effect was observed to be enhanced, becoming stronger as wettability, solubility, and hygroscopicity declined.

The double emulsions are comprised of two aqueous phases and one oil phase. These phases protect probiotics at different times. For example, the suspension of probiotics in the inner water phase, the outer water stage, and the oil layer offer protection to probiotics. The main method of preparation of the probiotics is through the two-step emulsification process. The first step is elementary of the water-oil emulsions, whereby polyglycerol polyricinoleate is applied for stabilization purposes. The second step involves the formation of the double emulsions through the combination of the outer water phase and the W1/O emulsions. The formation of the double emulsions and multilayer emulsions enhanced the protective effect of probiotics and, thus, a novel approach to the delivery of probiotics.

Nanoemulsion

Nanoemulsions comprise a colloidal particulate system that acts as a drug carrier molecule in the submicron size. Gao et al. (2021) assert that the size of nanoemulsions ranges between 10 and 1000 nm. The surface of the nanoemulsions is lipophilic and amorphous, containing a negative charge. As part of the delivery system of probiotics, nanoemulsion has been effective in enhancing the improved therapeutic efficacy of drugs and medications besides reducing damaging toxins and effects. Nanoemulsions have been effective in treating respiratory infections, cancer, vaccination, and enzyme replacement therapy in treating liver-related diseases. The oil-water emulsions are fabricated to produce the oil-water nanoemulsions, serving

as a delivery pathway for various categories of probiotics. These oil-water nanoemulsions have been classified as potential delivery systems due to their thermodynamic stability, which ensures effective delivery. Vaishanavi and Preetha (2021) indicated that stabilization of oil-water Nano emulsions is achieved through tween 80, gum Arabic, and soy protein isolate. The addition of Lactobacillus *delbrueckii subsp. Bulgaricusto* mixture provided enhanced stability to about 40 days of storage.

Beads

Beads act as a protective layer for viable probiotics and the delivery system. Beads prepared through alginate and calcium chloride were used in entrapping *Lactobacillus casei ATCC 393*. These beads were presumed to have a larger percentage of encapsulation of about 50%. One of the main weaknesses of this preparation is determining the optimal parameters in the preparation of beads. For example, although the shape of the beads was regular when exposed to adequate alginate concentrate, the size enlarged in low calcium content (Govender et al., 2014). The embedment of enterococcus in the alginate beads, especially in the injection of the dry-fermented sausages, revealed its potential to offer improved protection of probiotics during the ripening process. This shows that alginate acts as a natural polymer and raw material used in the fabrication of beads effective in the delivery of probiotics due to their unique chemical, physical, security, and biocompatibility features. However, commercial production of these beads is limited encase the freeze-drying method lowers the shelf life and survivability of probiotics.

Microcapsules

Microcapsules have the same size as beads, within a close range of microns, and act as physical barriers. Drawing on He et al. (2021), the encapsulation of Lactobacillus rhamnosus LMG25859, *Lactobacillus casei LMG6904T*, and *Lactobacillus acidophilus* LMG9433T with the application of low-methoxyl pecines showed that microcapsules contain the potential to protect probiotics, especially during fermentation. Also, the experiment showed the transformation of microcapsules to biofilm capsules after incubation, with a higher degree of probiotic protection. Another study by Jia et al. (2020) asserted that alginate and chitosan were used in the fabrication of microcapsules, which were applied in the encapsulation of probiotics, especially those made in the form of the vaccine. These vaccines are expressed through the use of the spring vermin carp virus G protein to achieve oral immunization, thus expanding the use of probiotics in various fields. On the other hand, the double-layered microcapsules, which are effective in encapsulating Lactobacillus casei LC2W, were made by mixing cellulose nanocrystals, whey protein, and sodium alginate. The addition of sodium alginate and calcium ions was used to aid

in the formation of the outer layer of the microcapsules, which contributed to the enhanced capability of the capsules in protecting probiotics. Good quality of many microcapsules is obtained through the freeze-dried powder, which enables them to have higher viability and improved efficiency of lactobacillus Plantarum as a result of the availability of the trace fish oil.

Microencapsulating technology has contributed to various innovative investigations and research regarding the delivery systems of probiotics through microcapsules. For example, a study by Zhao et al. (2022) applied the concept of dualism in fabricating dual-core microcapsules via electrostatic-driven micro fluids. The study established that the process enhanced the protection of Bacillus subtitles and Lactobacillus from harsh intestinal and gastric conditions. Also, it promoted the proliferation of these probiotics during the anaerobic fermentation process, where lactic acid is produced (Zhao et al., 2022). This strategy involved a lack of direct contact with the probiotics and enhancing their degradation to the form of microcapsules as an effective way of delivering probiotics. Electro-spraying is the current method used in the preparation of microcapsules because it provides a higher production yield with mild processing.

Hybrid Electrospun Nanofibers

The hybrid electrospun nanofibers, which are small compared to the microbeads, provide an effective probiotic delivery system as a silkworm cocoon. The species are in the form of biopolymer solution droplets whose surface areas can be removed via spinneret when exposed to higher electrostatic voltage. The alginate and whey protein concentrate are often used as biopolymers in the encapsulation matrices during the delivery of probiotics. Researchers and scientists have attracted increased attention to hybrid electrospun nanofibers in recent years. These studies led to the discovery of pullain and gum Arabic, which are used in their preparation. Also, the hybrid electrospun nanofibers have been preventing common encapsulation of lactobacillus strains such as *Lactobacillus casei* KLDS 1.0338, *Lactobacillus Plantarum* KLDS 1.0328, Lactobacillus acidophilus KLDS 1.0327, and *Lactobacillus rhamnosus* KLDS 1.0320 with the viability of up to 28 days (Gao et al., 2021). One advantage of hybrid electrospun nanofibers is that they are resistant to gastric conditions and act as heat intolerant because of their high melting points. It is also important to note that the exposure of hybrid electrospun nanofibers in low and room temperatures improves its applications, especially in the food industry. For example, using sodium alginate and polyvinyl alcohol in the fabrication of the hybrid nanofibers proved essential in encapsulating Lactobacillus fermented for 8 weeks and effectively protecting probiotics from harsh gastric and intestinal environments. Furthmore, studies conducted by Duman and Karadag (2021) showed that hybrid electrospun nanofibers have the feasibility of protecting probiotics, thus providing a guideline for further investigation.

Aerogel

The processing of aerogels has opened new ways of responding to the various technical issues of drug production and delivery which are also environmentally friendly. Aerogels are differentiated by their physical composition, including a low density of between 0.0001 and 0.2 g/cm^3, higher surface area, and porosity of higher than 90% of the mesoscale open pores (García-González et al., 2021). Aerogels are produced by replacing the inside fluid with a gas. Like hydrogels, aerogels are prepared using the 3D networks of organic polymers, the assembled colloidal composite material, and inorganic materials. The main differentiating feature between the hydrogels and the aerogels is the swelling degree of aerogels prepared using the dried networks. A permanent structure is obtained by stretching the self-assembled competent chains to levels that are hardly achieved through immersion in common solvents. Freeze-dying uses an original swelling attained by the hydrogel when exposed to the aqueous medium via a rapid water freezing process and then sublimed under low-pressure conditions (García-González et al., 2021). However, some forces, such as liquid-solid adhesive and liquid-gas surface, may contribute to the shrinking of pores and thus promote increased solid interaction. Another drawback in the preparation of aerogel is an expansion of the water during the freezing process, which may also contribute to the destruction of the aerogel structure. All these drawbacks can be prevented through solvents that prevent destructive surface tension. Further, some aerogels are produced through supercritical processing approaches, which encompass a range of changes in the liquid phases. Supercritical processing effectively prevents liquid-gas tension at the surface and reduces it. This helps in the prevention of the aerogel's original pores. Also, the supercritical drying approach is compatible with several solvents, allowing for the use of unsuitable poorly-soluble water materials when preparing hydrogels.

The use of interconnected pores, tunable mesh sizes, homogenous structures, and increased surface area is essential in investigating the potential of aerogel as a drug delivery system. Compared to pharmaceutical hydrogels, aerogels allow for smaller, faster loading of molecules, which are less constrained, enabling them to easily access various regions, especially the inner matrix, thus contributing to effective interactions with the polymer matrix. Some of the physical properties of aerogels make them among the most beneficial drug delivery systems via various parenteral and mucosal administration routes (García-González et al., 2021). Aerogels contain a higher capability in terms of the absorption of liquids, thus enhancing the effective regulation of exudates thus promoting a quick wound-healing process. The aerogels' solubility also promotes enhanced drug penetration through the dermis, depicting that aerogels are effective transdermal treatments. Aerogels are also attractive treatment methods for lung disease through pulmonary administration and labile biopharmaceuticals used in vaccination and gene therapy via systemic delivery. As a result of the great feasibility, modularity, and versatility of aerogels, they are increasingly produced on a large scale today as an exciting drug delivery system.

Food Conjugation

One of the common food conjugations takes place with biopolymers, whereby protein is the primary material used for developing various delivery systems. The conjugation process takes place through the Maillard reaction, which in recent years has received increased attention, especially regarding the encapsulation of flavors, volatile oils, and other bioactive compounds used mostly in the pharmaceutical and food industries (Nooshkam & Varidi, 2020). In the conjugation of protein polysaccharides, trostatic and steric polysaccharides are rendered, thus allowing for the attachment of the conjugated proteins to the hydrophobic surfaces. The Maillard reaction of food conjugation takes place in three main stages.

The first stage, the early stage, involves the formation of covalent bonds between the free amino group and the carbonyl group that reduces sugar to make a Schiff base and one molecule of water. Afterward, the Schiff base goes through the cyclization process, forming an N-substituted glycosylamine, a Lowy condensed product (Nooshkam & Varidi, 2020). The formed glycosylamine is then converted to stable 1-amino-1-deoxy-2-ketose from the aldose sugars. At this stage, the products are not caned, and capacity in the initial stage declines as the biological value of proteins increases.

The second stage is the intermediate state, shown by the degradation of the HRPs and the ARPs to form intermediate compounds through enolization routes 1, 2, and 2, and 3-enolization pathways at the original pH level of $\leq$7.0. at this pH, both the initial compounds undergo 1,2-enolization to produce hydroxymethyl furfural from either pentoses or hexoses. At the 2,3-enolization route, redoctones are produced, including fission products and 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (Nooshkam & Varidi, 2020). The reaction of these compounds with amino acids leads to the production and formation of aminoketones and aldehydes through the Strecker degradation pathway.

The final stage is where the Strecker degraded products, the fusion, and reductones go through the aldehyde-amine and aldol condensation to produce melanoidins and brown nitrogenous polymers (Nooshkam & Varidi, 2020). Although it has been reported that the existence of food melanoidins in daily diet, advanced stages of Maillard reaction contribute to developing diseases such as Alzheimer's and diabetes. However, in controlled conditions, the Maillard reaction can be effective in food conjugation by preventing the formation of harmful compounds in advanced stages.

Spider Webs

Unlike silkworm cocoons, spider webs are defined as a network to prevent some important probiotics from being destroyed by unfavorable external conditions. The spider webs act in the form of gel-like structures, which have the potential and capacity to impact the network as a way of entrapping viable probiotics. This

gel-like are classified into the hydrogels and the bigels food-grade delivery systems, which will be discussed in depth in the subsequent sections.

Hydrogels

Hydrogels are crosslinked structures that are classified into three different categories depending on their sizes. These include the macrogels, which are the largest, the microgels, and the nanogels. The macrogels serve as the largest form of hydrogels used to encapsulate probiotics. Deliberating on current research conducted by Zhang et al. (2023), using the oxidized high-amylose starch via 90% of the oxidation temperatures and initiating the fabrication through crosslinking of high-amylose starch macrogel proved effective in protecting the hydrophilic ingredients and the *lactobacillus paracasei*. Another study by Yuan et al. (2021) showed the effectiveness of using the enzymatic approach in fabricating the interpenetrating polymer network macrogels. This is improved with the sugar beet pectin and soy protein isolate, effectively encapsulating lactobacillus paracasei. Yuan et al. (2021) established that macrogels provide superior probiotic stabilization but less protection after the lyophilization process. This calls for effective regulation of the water content in the macrogels to ensure the successful delivery of probiotics.

To obtain the synergistic effect of probiotics, the macrogels are made of composite microgels through a small amount of ethanol, β-lactoglobulin, and propylene glycol alginate for co-delivering of curcumin and Lactobacillus rhamnosus GG. These systems effectively offered long-term protection of LGG and ATCC when exposed to low temperatures and UV light radiation during storage. The enforcement of the systems was attributed to the polysaccharide complexes, which can facilitate the efficiency of the system. Oxidized chitosan and *Bletilla striata* polysaccharide were also deployed in the fabrication of composite microgels used in loading Lactobacillus Plantarum, which is effective in the management of wounds due to its antibacterial properties. *Bletilla striata*, a traditional Chinese herb, serves as one of the novel materials used in the preparation of macrogels that provides better effects. Also, the gelatin crosslinking chemical was used to fabricate microgels, whereby glutaraldehyde was used as the crosslinking agent. Patarroyo et al. (2021) assert that ideal microgels were applied in Kluyveromyces lactis GG799. Then later, glutaraldehyde and graphene oxide were used to produce the double crosslinked microgels effective in Kluyveromyces lactis encapsulation.

On the other hand, the double-network macrogels, which consist of lactobacillus rhamnosus GG, are manufactured through the utilization of sodium alginate, which shrinks when immersed in gastric juice, and portrays bulging features when put in simulated intestinal fluid. Another study by Yuan et al. (2021) showed that macrogels prepared from sodium alginate stood out from silicon dioxide nanoparticles when exposed to protamine in either the metal-organic or york-shell frameworks while in the 2-methylimidazole zinc-salt. These substances proved effective in protecting encapsulated probiotics such as Bifidobacterium breve ATCC15700 in harmful tetracycline and gastric acid conditions. Macrogels are also prepared

through a self-cross-linking approach using the thiolated hyaluronic by encapsulating Lactobacillus rhamnosus ATCC 7469 (Gao et al., 2021). Microgels often bind and degrade the host when exposed to hydrogen sulfide produced by intestinal microorganisms. Consequently, macrogels provide an effective window for the encapsulation of probiotics, improving.

The microgels, another species of hydrogels, are smaller in size compared to the macrogels. Microgels can deliver probiotics just like macrogels. Gao et al. (2021) noted that *Bifidobacterium pseudocatenulatum* G7 was used to encapsulate the microgels by utilizing antacid and alginate, which provided enhanced protection of probiotics when exposed to the vitro gastric environment, especially in the small intestine. When microgels are fermented, they release probiotics under the colonic environment, revealing the targeted gut release implementation. However, there are few in Vivo experiments to prove this assumption. When nanoemulsion lipid droplets are added to the system, they provide enhanced protection of the probiotics when exposed under simulated gastric conditions. One of the best approaches to enhancing the effective delivery of probiotics is the composite of various systems. Another important microgel used in responding to gastrointestinal conditions is *Pediococcus pentosaceus* Li05, which can prevent the development of Clostridium difficile infections by enriching microbial gut diversity. Also, using microgels to encapsulate probiotics has proved effective in treating disease through the intake of certain foods, which is essential in replacing the traditional forms of treatment.

The nanogels are the slimmest species in the hydrogel classification. Although there is limited literature, available studies show that nonogels serve as important probiotic delivery systems (Gao et al., 2021). Ashoori et al. (2020) asserted that the embedment of *Bacillus subtilis* sp. *natto* ATCC 15245, *Lactobacillus fermentum* ATCC 9338, and Lactobacillus reuteri ATCC23272 in the chitosan nanogel, proved effective in enhancing the process of wound healing. This study also showed that nonogels, despite their size, can encapsulate probiotics. However, all the nano gels encapsulated probiotics are not orally delivered or administered, thus indicating the need for further research on the delivery systems of non-gel-loaded probiotics.

Bigels

Bigels, which are also known as hybrid gels, are creams that are comprised of oleogels and hydrogels. This category of gels is effective in delivery systems from hydrophobic ingredients and the hydrophilic. The concept of bigels was first advanced in 2008 by Almeida et al. (2008) and later followed by a range of examinations and investigations regarding the application and fabrication of bigels. Structuring the internal and external phases of the bigels is important in improving the stability of probiotics, especially when they are required to be stored for a long period. Bigels, unlike other multiphase systems such as emulsions, enhance the long-term stability of probiotics. The food-grade bigels are categorized into three main species based on the distribution of the species. These three species consist of bi-continuous, oleogel-in-hydrogel, and hydrogel-in-oleogel.

Several studies have been conducted to investigate the bigels. For instance, Behera et al. (2014) showed that polysaccharides, sunflower, and Sorbian monopalmitate, are important in protecting lactobacillus Plantarum 299v, especially in intestinal and gastric conditions. This study established that bigels are effective in the application of the delivery of probiotics. Another study by Bollom et al. (2021) showed that some bigels, such as whey protein, stearic acid, and soy lecithin concentrate, were used to protect probiotics such as lactobacillus acidophilus and bifidobacterium lactis, especially from the unfavorable environment which may harm and destroy their viability. However, the research found that bigels offered good protection to the Lactobacillus acidophilus probiotic compared to the Bifidobacterium lactis species. Although it is complex to distinguish the different protective attributes of bigels in different probiotics, this study is important in giving insight into the future, where scholars need to examine the applicable delivery systems for various probiotic genera. However, the study on bigels used in the delivery of probiotics is still scant, thus providing limited information about their delivery systems of probiotics.

Applications of Food-Grade Delivery System

Drinks and Ice-Creams

Probiotics have been effectively applied and approved in the production and manufacture of dairy products such as yogurts, which are classified as part of the functional foods that provide many health benefits to the human body. Gao et al. (2021) assert that over the years, the classification of yogurts as healthy foods was boosted when encapsulated probiotics were added to the yogurts. Ajloouni et al. (2021) showed that the bioaccessibility of Bifidobacterium lactis, BB-12, and Lactobacillus acidophilus during fermentation and LA-5 greatly multiplied in the colon. Adding encapsulated probiotics to the dairy products such as yogurts during fermentation is important in maintaining their viability. Other products, such as almond milk, coconut milk, and soymilk which are classified as plant milk substrates, also proved effective in the delivery of probiotics (Gao et al., 2021). This is because these substrates have the potential to tolerate lactose intolerance and provision of a high-cholesterol diet. These milk products also overcome protein allergy, which is common in cow milk. In substituting cow milk, other plant milk substitutes originate from plant products such as cashew nuts, walnut, buckwheat, peanut, and maize, whose mixtures have been applied in the delivery of probiotics. However, one issue with plant milk substitutes is their unpleasant flavor and nutritional value, making them less attractive to consumers than animal milk.

Probiotics can also be utilized in various vegetable and fruit juices, including aloe Vera drinks, pineapple juice, raspberry juice, cherry juice, and orange juice. In recent years, there has been an increased demand for natural vegetable and fruit juices due to their nutritious value and pleasant flavor. Lillo-Pérez et al. (2021) assert vegetable and fruit juices are essential in maintaining and retaining the

viability of probiotics. Fruits and vegetables contain additive nutrition, which is produced through the use of the cellular synthesis process. A larger percentage of the nutrients available in vegetables and fruits, such as antioxidants, fiber, minerals, and vitamins, are essential in supporting the growth of probiotics.

Besides dairy products, fruits, and vegetables, ice creams are also effective in delivering probiotics. Ice creams serve as one of the appropriate mechanisms to support the shelf-life of probiotics, especially when they are supposed to be stored for a long period (Gao et al., 2021). This shows that encapsulation plays a significant role in the delivery of probiotics when used in ice creams.

Cheeses and Butter

Another application of the food-grade delivery system of probiotics has been demonstrated through cheeses and butter. Gao et al. (2021) assert that cheeses qualify as the ideal food products essential in protecting probiotics due to the high-fat contents and pH levels present in cheeses. Also, unlike other food matrixes, cheeses contain low water and oxygen activity, which are essential in providing the nutrient and buffering capacity, which is important in protecting probiotics. A study by da Silva et al. (2021) showed that some probiotics, such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus,* are commonly used in the encapsulation of capsules. These same probiotics are also applied in the preparation of butter. The produced butter showed desirable applicability in supporting the viability of probiotics by enhancing their stability during long storage.

Capsules and Tablets

Capsules and tablets are among the commonly used and acceptable drugs administered in various forms to treat various conditions. Tablets and capsules are portable and dry products that have proved effective in the delivery of probiotics. Encapsulation of tablets and capsules has been a standard and feasible strategy for forming oral probiotic supplements. A study by Kumar et al. (2020) showed that tablets such as eudragit S100, guar gum, pectin, and 5-Fluorouracil mini tablets effective for the colon were manufactured and encapsulated with *Saccharomyces boulardii.* Encapsulation of tablets and capsules using various probiotics is effective in maintaining their viability in the long term.

Conclusion

With the current increase in lifestyle-related illnesses, consumers worldwide are more concerned about what they consume and taking value for their money. Nutrition-based behaviors have become the alternative source of treatment for

several lifestyles related illnesses. Probiotics are incorporated into both dairy and non-dairy-based foods to enhance their nutrition and health values, such foods may include yogurt, cheese, breakfast cereals, and sausages. The consumption of supplements and foods rich in probiotics is effective in aiding bodily functions. The delivery of probiotics has been a topic of interest for years and recently, there is an increase in developing delivery systems for Lactic Acid Bacteria which have the capability of delivering adequate amounts of bacteria and maintaining the functionality and viability of probiotic cells.

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Chapter 11
Using Bioprocesses and Biosystems for Environmental Protection, Microbial Detection, and Prevention in the Food Industry

Poornima Singh, Mohit Sharma, and Rashmi Rawat

Introduction

Biotechnology is defined as "the application of components, organisms, cells thereof, and molecular analogues for products and services through the merger of natural sciences and engineering" (Glick & Patten, 2022; Hernández-Arriaga et al., 2022). Biotechnology is adaptable and has been identified as a critical area that has significantly influenced different technologies which rely on the usage of various approaches in foodstuffs processing, agriculture, pharmaceutical, resource conservation, and environmental preservation (Fig. 11.1) (Goswami et al., 2022). This new era of technological changes has resulted in dramatical improvements in a variety of sectors (production of vitamins, drugs, interferon, steroids, fermentation products used as drink/food, energy from natural sources and waste, and genetic engineering applied to animals, plants, and humans) it can allow a totally original opportunity for the economical creation of existing and new products (Ranawat et al., 2022; Goswami et al., 2022). Besides, ecological worries spur the use of biotechnology not just for contamination treatment (disinfecting of soil, water, and air). yet additionally to forestall waste and contamination in any case, as well with respect to ecological friendly chemical synthesis and bio-monitoring.

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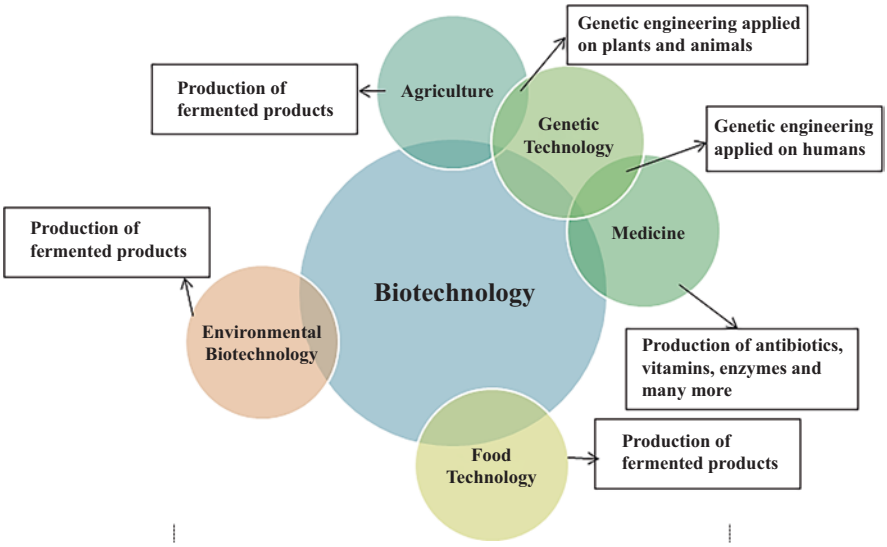

Fig. 11.1 Application of biotechnology in different fields

Nanotechnology is a developing subject in interdisciplinary study, particularly in biotechnology. Research in the nanoparticle is now unavoidable, not only because of its need but also because of the method of synthesis. The production, management, and application of materials in nanotechnology are measured in nanometers (De & Goswami, 2022; Li et al., 2021). Science has learned more and technology has improved as a result of the bridge of nanotechnology in the fields of medicinal plant biology and herbal medicine. There are several uses for nanotechnology, including in agriculture and medicine. Utilizing natural resources, nanotechnology in agriculture can be created to safeguard, produce, and safeguard livestock and crops (Silva et al., 2021; Kumari et al., 2022). However, the preponderance of the chemical processes used to manufacture nanoparticles entail the use of hazardous and damaging compounds that can pose biological dangers, and these chemical processes are not always healthy for the environment. It raises the demand for ecologically friendly improvements by utilizing green synthesis and other biological processes (Salem & Fouda, 2021). Using diverse plant extracts and materials to produce nanoparticles might sometimes be more advantageous than other biosynthetic techniques that need extremely complex procedures for maintaining microbial growth.

Nano-bioanalytical and nano-biosensing systems are certain outcomes of noteworthy advancements in nanotechnology and its applications in pharmaceuticals, foodstuffs, the atmosphere, and energy (Rezaei & Shirani, 2022; Mun'delanji et al., 2015). Nanotechnology contributes significantly to progress and originality by increasing sensitivity and enabling applications based on nanobiosensors and nanosensors (Shang et al., 2019). Traditional bioanalytical techniques are covered by biosensors, but nanobiosensors have fundamentally altered this field by offering

potential alternatives by reducing the need for traditional laboratory procedures and by offering advantages like quicker response times, robustness, improved sensitivity, and portability.

Bioprocessing and Biosensing

Bioprocessing is the process of producing valuable goods by utilizing a living thing—typically cells or cell components, viruses, or a complete organism. End products can range from algae-derived biofuels to medicines derived from mould, such as penicillin (Savchenko, 2017). Beer created from yeast is another instance of bioprocessing in action. This topic requires an understanding of a range of scientific fields, including chemistry, biology, microbiology, biochemistry, and chemical engineering. This is due to the variety of uses for bioprocessing and the complexity of the phases involved (Liu, 2020; Ghosh et al., 2016). Upstream and downstream bioprocessing are the two major components of bioprocessing. The upstream portion of bioprocessing involves the early stages of bioprocessing—identifying the organism to be generated, optimizing the conditions required for growth, and then growing and collecting this organism (John et al., 2020). The upstream process for biopharmaceuticals includes isolating the cell line to be generated, growing those cells to the scale required for the end product, and then harvesting those cells. Downstream bioprocesses include purifying the cells or other organisms collected after the upstream stage to create a finished product that fulfils high safety and quality standards (Rangle et al., 2020). Another important part of bioprocessing is bioprocess engineering, which entails optimizing the environment or system in which the organism inhabits to guarantee it can generate the desired output at the scale and quality required, yet at the lowest possible cost.

While the word "biosensor" was first used by Cammann, and the IUPAC later defined it as Engineering, chemistry, and biology must all work together to develop the materials, transducing devices, and immobilization techniques needed to create biosensors. Based on their processes, the materials employed in biosensors are divided into three groups: the bio-catalytic group, which comprises the set of bio-affinity, which comprised of antibodies, enzymes, nucleic acids, Isolated bacterial cells & microbes fonded group, which includes microorganisms' biosensors typically consists of biological components like an enzyme, an antibody, an organelle, a transducer, or an analyte (Sinha et al., 2020). These components are highly selective because they can be tailored to interact with an analyte in a particular way thanks to a biological recognition element on the sensor substrate that has a particular affinity for the molecule of interest. The biochemical sign is accordingly transformed into an electrical sign, either nonstop or intermittent, and afterward assessed when the natural material comes into contact with the proper transducer (Saxena et al., 2021; Sinha et al., 2020). A biological reaction is transformed into an electrical signal by biosensors, which are analytical tools. Recognizably, a biosensor must be extremely precise, unaffected by physical factors like pH and temperature, and reusable.

Biosensing and Micro-Nano devices

Frameworks for nano-biosensing and nanobioanalysis are undoubtedly the results of significant headways in the space of nanotechnology and its purposes in the pharmaceuticals, the atmosphere, foodstuffs, and energy fields. The advancement and innovation brought about by nanotechnology, which increases sensitivity and makes it possible to use nanosensors and nanobiosensors in applications, are significant (Table 11.1) (Rezaei & Shirani, 2022). By description, an instrument conveys chemical data of specific percentage value of component of the specimen to an analysis of the sample's whole composition into signals that may be used for analysis comprehended as chemical sensors. Bioactive components like, an bioactive-enzyme, antibodies, or a nucleic acids sequence, are integrated with the relevant bodily transducer to assemble a quantifiable sign that is symmetrical to the amount of a chemical component present in a specimen. This type of analytical device is known as a "biosensor." Nano biosensors, Specifically, are transducers in view of nanomaterials with actual control at the nanoscales. (Malik et al., 2013; Rezaei & Shirani, 2022).

By utlizing continually incorporated, small, multiplexed nanosensors to detect and analyze target molecules, nano-biosensing and nano-bioanalysis can give highly sensitive and selective detection limits. This was made achievable by the disclosure, handling, and utilization of materials for the making of devices, whose morphological qualities give the ideal aversion to locate at the nanoscale. Traditional bioanalytical techniques are covered by biosensors, however, nanobiosensors have fundamentally altered this field by offering viable substitutes by minimizing traditional laboratory procedures as well as benefits including quick reaction times, enhanced sensitivity, resilience, and portability (Srivastava et al., 2018; Rezaei & Shirani, 2022).

Nanotechnology

Nanomaterials are substances with dimensions between 1 and 100 nm, and they are a special gift that nanotechnology has given to society. Nanomaterials have gained significant attraction in contemporary years due to their outstanding electrical, mechanical, and visual capabilities due to their nanoscale size & the best blend of surface and volume components within the general way of behaving (Holzinger et al., 2014; Rezaei & Shirani, 2022).

Table 11.1 The characterization of nanomaterial with its dimensions

S. no	Nanomaterial	Dimension	Size range (nm)
1.	Nanoparticle	3-D	1–100
2.	Nanowire/ nanotubes	2-D	1–100
3.	Nanofibers	2-D	50–300
4.	Nanofilm	1-D	1–100
5.	Nanoplates	2-D	1–100

Fullerene, graphene, carbon nanotubes, and carbon dots are illustrations of metal-composed nanoparticles, whereas the best examples of metal-composed nanoparticles are nanorods, nanowires, quantum dots & oxide nanoparticles. It is ideal to divide nanomaterials used for nano-bioanalytical and nano-biosensing applications into these two main groups (QDs). Fundamental aspects of carbon doped nanomaterials in the zero to three dimensions have made them possibly helpful for the creation of cutting-edge nano-bioanalytical and nano-biosensing technologies. New methods and opportunities for detecting and analyzing target molecules have been made possible by the use of nanomaterials such as carbon in biosensors. (Kour et al., 2020; Rezaei & Shirani, 2022).

A class of functional substances known as metal nanoparticles has distinct chemical and physical characteristics that are especially characterised by their dimensions, form, range, and design. Innovation of metal nanoparticles and their use in numerous fields, including electronics, sensing, catalysis, and medicine, has seen significant advancements. To address the needs for the finding of more accurate and favourably liable biomolecules; metal based nanoparticles plays paramount role in the formation of visionary biosensors and/or modifications in existing biosensing techniques. Multiple biosensors, such as (1) nano-biosensors for illness diagnosis, (2) Probes for in-cell following, Vivo imaging/ detecting, and surveillance of illness aetiology or therapeutic surveillance and Additionally tools have been developed at nanoscale (Baptista et al., 2011; Zhao et al., 2011). In the field of biosensing, such dignified metals as gold and silverware proffer remarkable and concentrated optical attributes (Springer et al., 2017).

Biosensor

The study and development of nanobiosensors are based on an understanding of the biosensing concept. A biosensor is a sensing device or measurement system that is intended to measure a substance using interactions biologically and to translate the results into a readable form using electromechanical interpretation and transduction (Rezaei & Shirani, 2022). There are three fundamental parts to every biosensor: a bioreceptor, a transducer, and a detector (Fig. 11.2). During activity, the bioreceptor, which is situated outwardly of the biosensor, comes into contact with the objective analyte. Target analytes are captured by bioreceptors with great selectivity and specificity (Koyun et al., 2012). Bioactive-Enzymes (Zhao et al., 2017), Antibodies (Kim et al., 2008), Total cells (Han et al., 2018), Aptamers (Kim et al., 2016), and Deoxyribonucleic acid (Li et al., 2010) are a few of the typical bioreceptors utilized to create biosensors. The biorecognition component is typically absorbed into the sampling panel of the biosensor to bring out the preparation. Sensitivity and selectivity must therefore be maintained by the techniques used to connect the biorecognition component to the biosensor. The most widely recognized strategies for immobilizing biorecognition parts are adsorption, capture, covalent holding, microencapsulation, and cross-connecting (Luong et al., 2008; Sassolas et al., 2012; Datta

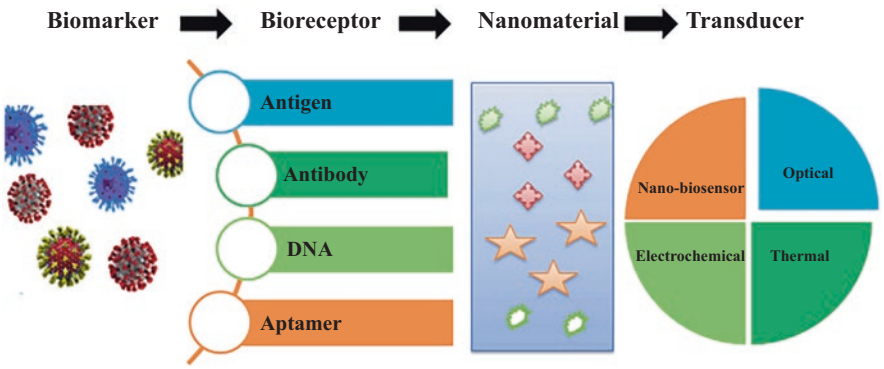

Fig. 11.2 Pictorial representation for working the biosensor

et al., 2013). The purpose of immobilization is to (1) repeatedly employ the biosensor; (2) Consistently screen the analytes in streaming examples, for example, natural liquids, ecological examples containing follow levels of target atoms, or bioreactor liquids; (3) enhance the sensitivity and reproducibility of biosensor performance by developing the biorecognition unit, and (4) make the immobilization strategy straightforward and versatile. The collaboration between a bio-analyte and its matching bioreceptor is generally changed over into an electrical structure continuously part of a biosensor, the transducer framework. A transducer, as its name suggests, effectively transforms one type of energy into the other. The first source of energy is biological in origin, whereas the second is often electrical due to the exact connection between the bioanalyte and the bioreceptor. The electronic indication from the transducer is shipped off the third component of a biosensor which is the indicator, and enhances adequately with the goal that the going with reaction can be perused and accurately examined.

Bioanalyses System

The preparation and detection of samples both heavily utilize bioanalysis systems. A bioanalysis system will first pre-treat and/or modify the recognition elements, then it will modify and/or treat the substrate surface before adding the biological recognition element, and last it will add the target analyte. The bioanalysis approach considers the classification of tools utilized & the construction of individually merged sophisticated segments for the analysis. (Rezaei & Shirani, 2022). Recently, bioanalytical researchers' nanomaterials are used for sample preparation. In extraction methods like micro-extraction, solid-phase extraction, and filtration, several nanomaterials have been used. An alternate sample preparation method to liquid-liquid extraction is solid-phase extraction (SPE), which can use less solvent overall. SPE has been used for many years to remove target analytes by pre-concentrate

from various matrices. In this methodology, the sorbent is allocated within discs, micro-columns, cartridges. Compounds based on silica, such as C18 bound silica, are typical SPE sorbents. The shrinking of SPE cartridges directed towards the preface of a brand-new microextraction procedure comprehended as microextraction by packed sorbent (MEPS) (Abdel-Rehim, 2004). Microsyringes are used in MEPS in place of SPE cartridges. The adsorbent material can be employed within the needle as a cartridge or a plug and closely compacted in the needle's barrel. This approach is specifically valuable for completely automatic online analyses. The integral specimen volume also vastly diminished to a fewer ml. For bioanalytical uses, much more advanced sorbents can be packed and used. This strategy has an equal chance of being used online as other absorbents, including established adsorbent materials.

QDs' distinctive optical & electrical aspects have led to surface modification for usage in bioimaging and biomedicine (Clapp et al., 2006). In general, bonding (covalent and non-covalent) is the two categories into which the methods for altering QD surfaces are divided. Specifically, in a non-covalent procedure, that comprises electrostatic interchanges. Organophosphorus hydrolase (OPH) was proficiently bioconjugated with adversely charged CdSe/ZnS QDs via electrostatic cooperations, which likewise created emphatically charged protein side chains and NH2 end groups linkages (Ji et al., 2005).

Due to their exceptional optical and electrical capabilities, Quantum dots, which are metal doped Nanoparticles, have been engaged in multiplexing examination, fluid and solid stage designs & trace investigation of inorganic substances. Based on the changes in the fluorescence characteristic, several techniques have been devised to identify Ag (I). Relying on the concentration of Ag (I), various fluorescence responses are witnessed (Xia et al., 2008). According to reports, QD fluorescence is enhanced by low Ag (I) concentrations and vice versa. According to the findings, particle size is important because most trapping flaws in tiny particles originate on surfaces that may passivate to increase fluorescence. In systems based on QDs, fluorescence quenching-based techniques are the most popular ones for detecting Pb (II).

Use of Bioprocesses and Biosystems

Two key instruments for promoting social welfare and economic prosperity are biotechnology and bioprocesses. As they create processes employing genetics biology, synthetic biology, molecular biology, and competitive biotechnological goods, as alternatives to chemical-based applications, the academic, industrial, and governmental sectors are certain to run into technical issues. The interaction between a bioanalyte and its matching bioreceptor is mostly transformed into an electrical state by the dual element of a biosensor, the transducer (Croughan et al., 2015; Shong et al., 2012). Improved bioprocesses are constantly needed in the biopharmaceutical sector to handle new regulatory requirements, quality control requirements, manufacturing issues with cell culture titration, biological products, and the

creation of biosimilars (Whitford, 2013; Cramer & Holstein, 2011). Biotechnology has many purposes, for example, food handling, planning, and enhancement to raise supplement input; process advancement for monoclonal antibody response filtration for the treatment of different cases; assessment of host cell proteins (HCPs) and improvement of microorganisms for the handling and transformation of biomass into biofuels, the making of helpful antibodies, and the production of hematopoietic stem cells (HSCs) for restorative applications (Barragán-Ocaña et al., 2020).

Environmental Protection

By using biotechnology to (bio)treat/(bio)remediate historic pollution as well as address it through pollution prevention and control techniques, environmental dangers and hazards brought on by accumulated hazardous chemicals or other waste and pollutants might be diminished or eliminated. Pollution stemming from present industrial practices could also be minimized. The US Environmental Protection Agency (USEPA) characterizes bioremediation as "a directed or spontaneous approach wherein microbiological exercises are exploited to breakdown or change pollutants into nontoxic forms, ultimately remediating or removing environmental pollution" (Gavrilescu, 2010). For the monitoring and detection of diverse environmental contaminants, biosensors such as genosensors, aptasensors, immunosensors, and enzymatic biosensors have been described shown in Table 11.2. These biosensors use nucleic acids, antibodies, enzymes, and aptamers as the appropriate recognition components (Justino et al., 2017).

Identification of Pesticides

Because of their broad use in the environment, pesticides are among the harmful substances. For example, the group of pesticides comprehended as organophosphorus insecticides, which are ordinarily utilized in agriculture, represent a critical risk to the environment because of their high harmfulness. Thus, insightful techniques without extensive sample pre-treatment, including biosensors, have been made for their observation and identification (Justino et al., 2017). For the finding of organophosphorus pesticides, dispensable amperometric enzymatic (acetylcholinesterase) biosensors with cysteamine self-gathered surface on gold screen printed cathodes were proposed utilizing paraoxon as the prototype (Lang et al., 2016; Arduini et al., 2013). The detection limit of disposable biosensors falls between 2 ppb to 40 ppb with a sensitivity of 113 A mM cm^{-2}. The scientific presentation was significant because of the profoundly orientated enzyme immobilization employing oneself collected monolayer. Recovery yields of 98.3% (n = 3) were kept after tests in stream water examinations spiked with 10 ppb of paraoxon, demonstrating the proficiency of such enzymatic biosensors (Guo et al., 2017). Other biosensors for the identification of paraoxon in actual water specimens have been examined, including

Table 11.2 Application of various types of biosensors and its role in environment protection

S. No	Pollutant detection	Type of biosensor	Recognizing element	Detection limit	References
1.	Paraoxon	Electrochemical	Enzymes	Approx. 40 ppb	Arduini et al. (2013)
2.				Approx. 30 μgL^{-1}	Hassani et al. (2017)
3.				1 nm–5μM	Lang et al. (2016)
4.	Methyl parathion			0.5–1000μgML^{-1}	Zhao et al. (2013)
5.				0–1500 μgML^{-1}	Mishra et al. (2017)
6.	Chlorpyrifos			0.01–0.1μM	Mayorga-Martinez et al. (2014)
7.	Carbaryl			1–9 μM	Santos et al. (2015)
8.	*E. coli*	Optical	Histidine	–	Yilmaz et al. (2015)
9.	*Bacillus subtilis*	Electrochemical	Antibodies	10^9–10^{10} CFU/mL	Yoo et al. (2017)
10.	Okadaic acid	Optical	Antibodies	–	McNamee et al. (2013)
11.	Bisphenol	Optical	Aptamers	1–10000 $ngmL^{-1}$	Ragavan et al. (2013)

an amperometric acetylcholinesterase biosensor assembled from gold nanoparticles. The most extreme buildup level showed in the European Association pesticides data set was not exactly the identification furthest reaches of amperometric and colourimetric biosensors, which were 4.8 ppb (18 nM) and 0.8 nM. The amperometric biosensor was utilized to recognize paraoxon in specimens of stream water and ocean water with recuperations of 96–98%, while the colourimetric biosensor was utilized to distinguish paraoxon in agriculture irrigation system, water with recuperations rate of 88–100%. (Laws of the EU, 2017).

Identification of Toxins

Since cyanobacteria bloom caused by eutrophication of aquatic systems creates harmful toxins such as microcystins and brevetoxins, reliable and affordable technologies are required for the early identification of such toxins. Utilizing electrodes assembled of gold, operationalized with a cysteamine self-gathered monolayer, an electrochemical aptasensor was utilized to delicately recognize brevetoxin-2, a marine neurotoxin (Eissa et al., 2015). A constraint of identification of 106 pg mL^{-1} was accomplished, and brevetoxin-2 exhibited high selectivity against diverse toxins from a periodic class, including okadaic corrosive and microcystins. By breaking down shellfish, the reasonability of the aptasensor to identify brevetoxin-2 in

genuine specimens was conducted. Great recuperations rate of 102–110% was accomplished, showing that there were no obstructions from the shellfish framework on the aptasensor sensors. (McNamee et al., 2013). Furthermore, the utilization of biosensors for the observation of toxin okadaic acid in algal, saltwater and shellfish specimens were conveyed. A multiplex surface plasmon biosensor was implied for okadaic investigation in algal and saltwater specimens, as well as for the observation of saxitoxin and domoic acid. Okadaic acid was observed in algal cells utilising a concise specimen preparation approach that affected employing glass beads to lyse the cells and release the toxins, heeded by centrifuging and purifying the extract.Toxins Saxitoxin (0.82 ng/ml), okadaic acid (0.36 ng/ml), and domoic acid (1.66 ng/ml) had been detected in the observation respectively (Pan et al., 2017).

Identification of Pathogens

Pathogens can designate a significant hazard to human health when present in enveloping matrices, particularly water compartments, and certain biosensors have lately been offered for their environmental monitoring. For example, surface plasmon resonance based optical biosensors that can identify metabolically dynamic Legionella pneumophila in experimenting natural water trials have been recommended (Foudeh et al., 2015; Enrico et al., 2013). The uncovering of bacterial RNA by the probe employed to identify RNA is mounted on the biochip gold surface was the basis for the detection concept in one study (Foudeh et al., 2015). The biosensing gadget seems suitable for the effective discovery of microorganisms in the possibility of 10^4–10^8 CFU/ ml, as shown by the utilization of streptavidin-formed quantum dots (Foudeh et al., 2015). In the second experiment a self-assembled protein a monolayer and an anti-*L. the pneumophila* antibody solution was used to functionalize the gold substrate (Enrico et al., 2013). Because no labelling was required and antibody immobilization on the biosensor surface by protein, a limit of proportions of 103 CFU mL-1 was acquired and the biosensor was capable of determinate *L. pneumophila* in tainted water specimens in 30–45 min (Enrico et al., 2013).

The results showed strong selectivity, a good detection limit (10^4 CFU mL^{-1}), and acceptable stability. *Salmonella, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis,* and *Aeromonas* were observed to see if the selectivity interfered with them. During the shelf-life study, detection of L. pneumophila was observed (10 CFU mL^{-1}) by plasmon resonance (Meneghello et al., 2017).

Others

The requirement for novel, fast, and reliable insightful methodologies have emerged because of the continuous event of risky algal blooms. Biosensors for recognizing algal RNA have been created because of the unprecedented particularity and responsiveness of nucleotide tests to their related restricting accomplices (Orozco et al., 2016; McPartlin et al., 2017). Lately, it was proclaimed that an electrochemical

genosensor casted on a screen-mounted gold electrode could inspect the RNA of 13 unsafe algae species. The genosensor was competent to isolate RNA targets from ocean water specimens. (Orozco et al., 2016). Environmental biosensors have also been utilised to determinate halogenated combinations. For example, a fluorescence-based enzymatic biosensor was designed to observe 1,2-dichloroethane, 1,2,3-trichloropropane, and hexachlorocyclohexane in ocean water specimens within pH ranges of 3–10 and controlled temperatures 5–50 °C (Bidmanova et al., 2016). Detection limits for beforementioned halogenated compounds were found to be 2.7, 1.4, and 12.1 mg/L, respectively. Biosensor was operated for speedy observation of 1,2-dichloroethane contamination in seawater specimens during real-world testing and can map the distribution of the contamination using GPS (Bidmanova et al., 2016).

Microbial Detection

The most prevalent foodborne pathogens, which yearly infect millions of people, are *Salmonella species, Campylobacter species, Listeria monocytogenes,* and *E Coli* O157 (Finn et al., 2013; Alvarez-Ordóez et al., 2018; Lamas et al., 2018; McCabe et al., 2019). Fever, cerebral pain, queasiness, regurgitating, stomach inconvenience and infrequently organ failure and demise are among the mild to severe symptoms. Responsive joint pain and Guillain Barré condition are two extreme sequelae that some foodborne diseases, for example, Campylobacter spp., can produce (Keithlin et al., 2014). Foods like eggs, poultry, and other commodities with animal origins, as well as those with them as the major ingredients are frequently connected to outbursts of foodborne illness (Ma et al., 2014). Heterogeneous distribution of illnesses rendering microorganisms, stress experienced by the microorganisms during food handling, and nontargeted microorganisms' presence from the ordinary microbiota (*Staphylococcus*, *Pseudomonas*, *Enterobacteriaceae*, *Bacillus*, *Acinetobacter*, and yeasts), particularly in natural food varieties, make fostering a strategy to screen food tests especially problematic (Varghese et al., 2016).

Due to their great heterogeneity and potential compatibility issues with the analytical procedures, food samples frequently need pre-treatments. Further, the mark microorganisms are generally present at remarkably inferior concentrations; in these occurrences, pooling of specimens may be feasible to boost up the analysis. Preparing food specimens for preanalytical testing has the subsequent goals: withdrawing mark pathogens/impurities from the food matrix, stimulating their ratios (in a few cases), isolating them from microbes matrix that are not targeted, and excluding inhibitory chemicals. Sequencing & molecular techniques, which are marked susceptible to inhibitory substances in the specimen, the later operation is extremely paramount. Food is oftentimes solely partly analysed during a microbiological review; just a representative specimen is taken into account for the analysis. The subsequent available measures must be taken into account when constructing a unexplored detection technique:

Microbial detection	Conventional method	ISO method
	Rapid method	Immunological method Molecular method Sequencing method
	Spectroscopic techniques	Near infrared spectroscopy Raman spectroscopy Biosensors Meta data profiling

Fig. 11.3 Different methods for detection of microbial load

(1) Enhanced specificity, detection at the lower limit, and sensitiveness; (2) Adaptable, making it functional by non-professional; (3) Improved observation time; (4) affordable; (5) Improved capacity and industrialisation, and (6) validation against tried-and-true methods. The detection practices are conferred below and illustrated in Fig. 11.3.

Conventional Method

Traditional culture techniques, such as preparation of specimens, enrichment, serial dilution, count, and separateness of individual species colonies for succeeding reports, are the foundation of the traditional procedure utilised to specify & detect foodborne bacteria. According to the sampling/characteristic targets, the label established on the different characteristics using a range of strategies, as further explained below.

Commonly, total plate count or any agar-based medium can be utilised for the total viable count of bacteria in non-specific cultures (TVC). For the enumeration of certain groups of bacteria and pathogens, selective and/or differential media are employed (Gracias & McKillip, 2004). Utilizing a mixture of combinations, for example Gram stain & biochemical or serological tests, each morphologically diverse colony retrieved from the specimen, in particular catalase or potentially oxidase activity (distinct colony, dimension & colour as assessed by a human spectator) and additionally inspected for pathogenicity or potentinal role in food spoilage. (Castro et al., 2017; Gracias & McKillip, 2004). Because of their reliability, usefulness, sensitiveness, and broad-spectrum applications, traditional culturing methods

nevertheless labelled as the most acceptable. They are yet a prerequisite for count and detection, defining validating, and viability phenotype projections established on genomic breakdown. The prevalence of ISO measures for estimating security and sanitary indicators (moulds, *Enterobacteriaceae, yeasts* & lactic acid bacteria) and exposing pathogens (*Listeria, E. coli*, *Staphylococcus, Salmonella*) are founded on standard culturing approaches, and the regulatory boards specified permissible limits for individual category.

Rapid Method

Rapid testing may be qualitative, identifying the presence of microbial contamination, for instance, by measuring changes in pressure, impedance, or CO2 concentration (Bancalari et al., 2016; Dheilly et al., 2008). The impedimetric approach uses conductance changes caused by bacterial growth to quickly identify microorganisms in samples. In distinction to execute impedance technology, which utilises the shift in conductivity of a fluid culture medium as a determinating framework, indirect impedentiometry estimates the transformation in electrical conductivity of a retort solution, which emerges as a consequence of the absorption of gases from the immunised bacterial culture.

In the depth of food microbiology, impedance has mostly been utilised to determinate and measure Enterobacteriaceae. This procedure's primary limitation is that it can only be effectively used when working with extremely identical specimens, as it brings a lot of effort to optimize the process and calibrate individual category. (Ferone et al., 2020). Furthermore, if the microorganisms have been exposed to aspects that drive sub-lethal damage to the bacteria, this method is not fit for estimating the total bacterial count.

Spectroscopic Methods

The investigation of matter established on how it interacts with electromagnetic radiation is comprehended as spectroscopy. There are numerous distinctive spectroscopic techniques available to manage a sort of analytical issues. The approaches vary relying on the species being investigated (atomic/ molecular spectroscopy), radiation-matter interaction noticed (like emission, absorption), and the extent of the electromagnetic range being analysed. For analytical investigations, spectroscopic approaches have been employed in practically all technological sectors of engineering, including life sciences (Ferone et al., 2020). Diverse absorption spectra can be created by the distinct macromolecular makeup of bacterial cells (i.e., proteins, nucleic acids, Fats & Carbohydrates). Nevertheless, because of the majority of microbes have extremely identical spectra due to the slight differences in their chemical makeup. To quantify and differentiate microorganisms, spectroscopic methods must be paired with spectral preprocessing and other chemometric methods. Partial least square regression (PLSR), artificial neural network (ANN) and

support vector machine (SVM) and stepwise multiple linear regression (SMR) are the most frequently utilized chemometric approaches for quantitative investigation.

Prevention in Food Industries

With the development of nanotechnology, it is now possible to influence matter at the supramolecular or atomic level, leading to a wealth of discoveries and uses for nanoscale materials. As a result, key international industries have experienced a progressive innovation leap thanks to nanotechnology. Despite being a multi-trillion-dollar business, the food and beverage industry meets the basic needs of the majority of people. The Industrial Revolution 4.0 is coming, and with it will come a speed-up toward an autonomous and effective process. To make use of the extended versatility of nanoscience to improve the production, packaging, security, aroma, and quality management of their produce. Food conglomerates are drawn to and facilitated to engage in nanotechnological study (Jideani et al., 2020). As a outcome, In recent years, Numerous nanotech products are utilised in the food supply and are demonstrated in Table 11.3. The preponderance of the measures is intended for use by others. These possess biodegradable and environmentally friendly nanomaterials, as well as novel packaging techniques that preserves & monitors the food process until it gets consumes. Antimicrobial packaging, nano based sensors and active packaging techniques for barrier protection against microorganisms employed for protection. Nanoparticles are also incorporated into the food products such as the encapsulation of functional materials in supplements. Nanomaterials-based techniques, such as nanosensors and nanodipsticks, are innovations that combine and miniaturize complementing approaches. These have flared an increase in research into and the innovation of mobile smartphone-based sensor arrays for usefulness in the lab and outdoors. As a consequence, nanotechnology is incredibly valuable for the food industry and can suffice the gap as a feasible explanation to unsolved food research problems. Nonetheless, there remains a significant transitional obstacle for underdeveloped nations that depend heavily on food sources. Economic conditions have an mark on domestic and multinational aids for analysis and development, which are yet in their premature phases (Chaudhry & Castle, 2011). Safety concerns are another major obstacle to their deployment since nanoparticles'physiochemical characteristics differ greatly from those of their macro-counterparts.

Nano-material Based Sensors in Food Analysis

Nanomaterials have measured between 1 and 100 nm. The tested nanomaterial systems, according to Mustafa & Andreescu (2020), possess organic-based (mainly biosensors, with periodic cases of biosynthesized nanoparticles and organic

Table 11.3 Application of biosensors in various food segments

S. No	Sample	Application	Biosensor	References
1.	Apple	Pesticides and heavy metals	Electrochemical biosensor	Chen et al. (2021)
2.	Apple, broccoli, and cabbage	Pesticides and heavy metals	Electrochemical biosensor	Cesarino et al. (2012)
3.	Mandarin	Infection and diseases	Electric nose	Park et al. (2015)
4.	Apples, kiwi, and pear	Ripening and maturity	Electrochemical ethylene sensor	Jia et al. (2016)
5.	Peaches	Infection and diseases	Electric nose	Liu et al. (2018)
6.	Fruits	Pesticides and heavy metals	Photo-electrochemical biosensor	Li et al. (2015)
7.	Vegetables	Pesticides and heavy metals	Conductometric Biosensor	Mulyasuryani and Dofir (2014)
8.	Apple	Defect detection	HSI and MSI	Zhang et al. (2018)
9.	Tomato	Insect infection	MSI	Mireei et al. (2017)
10	Apple	Quality determination and control	Optical biosensor	Saeys et al. (2008)
11.	Apple	Shelf-life assessment	MOS (prototype)	Trirongjitmoah et al. (2015)
12.	Peanut	Allergen	Aptamer based sensors	Peeters et al. (2014)
13.	Wheat	Allergen	Electrochemical	Ontiveros et al. (2017)
14.	Chicken	*Salmonella*	Optical	Zhang et al. (2018)
15.	Pork	Allergen	Calorimetric	Kuswandi et al. (2017)

product), inorganic (oxides metal, and compounded metal), and hybrid composites that merge both organic and inorganic substances (Majeed et al., 2013). In spans of how they operate, conventional sensor displays lean on molecular pattern recognition with customisable selectivity (biochemical) or electrochemical transduction, which mainly aims at volatile substances (Mustafa & Andreescu, 2020). The latest and progressing improvements in sensors are in optical biosensors established on surface plasmon resonance and colour change (SPR). A more careful history of the improvement of nanomaterial-based optical biosensors, with an emphasis on the joining of refractometric plans into the sensors, might be tracked down in this significant subject here (Kobun et al., 2015). According to reports, allergies, pollutants, pathogens, adulterants, minerals, and other foreign compounds that are judged dangerous for human consumption can be identified and detected using nanotech analytical procedures. Gold nanoparticles (AuNPs) are much of the time utilized in frameworks due to their momentous SPR abilities & silver nanoparticles (AgNPs)

because of their additional antibacterial property. The calibrating system of nanoparticle characteristics has been expanded over the course of the years by progressions nearby, prompting different structures and dimensions of Au/AgNPs for a more successful restricting cooperation towards target particles. Albeit metal oxides are all the more often found in the advanced food business as endorsed added substances, (for example, TiO2 for food colouring), they are additionally presumed to be significant for examination (Fe2O3 for its ferromagnetic and electromagnetic acceptance abilities) (He et al., 2019).

Detection of Allergen

Allergens in food are biological substances (mainly proteins) that can unintentionally provoke immune responses and display symptoms in a range of life-threatening degrees and can induce immunological reactions in people who consume or come into contact with them (Flanagan, 2014). The presence of food allergens in the final stage of production can be attributed to two main factors: (1) naturally found proteins in the raw ingredients, such as glycinin in soybeans, ovalbumin in eggs, and sometimes diary goods, etc. (2) small amounts of allergens in connection with different foodstuffs in the exact production line of industry (Rai & Bai, 2017).

In newly published modified biosensors, fullerene nanoparticles and protease enzymes were merged for a brief and rapid identification of allergic substances in which kernels contains gluten (Ontiveros et al., 2017). Gliadin is the offender after gluten allergies, which direct to Celiac disorder, an untreatable illness (Checkin et al., 2016). A biosensor and electrochemical chronoamperometry are combined. Protease enzymes are entrapped on carbon electrodes, while the latest modifications of fullerene nanoparticles in the detector raise both selectivity and sensitiveness. The mdified biosensor has detection limit up to 0.56 mg/L and can identify gluten with a limit up to 8.4 mg/L under ideal conditions.

For security of customers who are allergic, peanuts have drawn a lot of attention for the identification of their allergens (such as arachin h1, arachin h2, and arachin h6). The detection of arachin h1 has become more accurate recently as more reported nanosensors. Peeters et al. (2014) investigated and designed aptamer-based detectors augmented with gold nanoparticles for the prosperous application of carbodiimide chemistry and covalently bound for examination of arachin h1. Impedance spectroscopy has detection range of 1–250 nM and is utilised to carry out real-time measurement of arachin h1's binding affinity. By employing second nanoparticle alteration Fish parvalbumin & Pen (a-1) were analysed (Jiang et al., 2015). To build the sign from the limiting connections of antibodies-allergen; silicon dioxide covering was applied to Fe3O4 nanoparticles encased in liposomes. Fish parvalbumin and shellfish Pen a 1 both had lower limits of detection up to 0.17 g/mL and 0.02 g/mL.

Identification of Pathogens

Food- and drink-borne illnesses are well known for being spread by tainted products containing germs and parasites. As indicated by the information from the WHO Foodborne Infection board and by the study of disease transmission Reference Group (FERG), 48 million people have become sick because of different microbes, such as *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* (Havelaar et al., 2015). Three criteria can be used to classify the difficulties and restrictions associated with integrating modern nano-sensing of harmful pollutants into the analytical climate: Real-time PCR must be sensitive enough to identify pathogens in low concentrations, and it must also incorporate pretreatment steps for future large-scale sensing. Ultimately, it must undoubtedly differentiate between living and non-living pathogens in edibles to dodge overestimating sickness hazards, which can have severe repercussions (Shen et al., 2021).

MnFe2O4 magnetic NPs-AuNP surface composite (AuMNPs) has been utilised to identify *S. aureus* and its toxicants (Wang et al., 2018). The adversely charged AuNPs were then electrostatically adsorbable on the outer layer of the magnetic NP centers after the polyethyleneimine coating and the magnetic NP centers had been formed. Great band intensity was visible in SERS data with a LOD of 10 cells/mL. Another work used a paper-based biosensor to combine magnetic nanobeads with a selected *S. aureus* peptide sequence (Suaifan et al., 2017). With the bare eye, observable colour differences induced by nanobead-peptide template dissociations could be witnessed, and the consequences were said to bring place fast. When incorporating AuNPs with a enduring magnet to exaggerate the magnetic signs of a screen-bounded carbon electrode, seperate subspecies of *Salmonella enterica* was also recognized. (Afonso et al., 2013). A LOD of 143 cells/mL of bacteria was successfully separated and isolated thanks to the conjugation of the capture antibodies in skimmed milk.

Detection of Adulterant

Consumers are exposed to substantial health risks when foods and drinks are adulterated. This happens when essential ingredients are removed or when inexpensive, subpar ones are added, which reduces the quality and safety of the product (Jha, 2016). Nearly all adulterants formerly used in the food sector have been categorized and outlawed by their harmful nature. Real-time applications are constrained by the high cost and complexity of techniques like HPLC, ELISA, and RT-PCR that need pre-treatments. Consequently, there is an enormous opportunity for novel countermeasures research using nanotechnology. Meatballs and other processed meat items are popular in both Asian and European cuisines. As per study conducted by Stephen and Chen (2016), hog meat may have been employed as an adulteration in processed beef and chicken meatballs to diminish the cost of the uncooked materials. When it comes to health, consuming too much-processed meat raises cholesterol and fat levels, which causes chronic illnesses including type I diabetes and

myocardial infarction. Due to ingesting swine serum albumins and subsequent IgE-mediation, unusual situations may result in allergic responses to pork (Wilson & Platts-Mills, 2018). On the other side, from a religious perspective, such as the Halal dietary rules, the adulteration and ingestion of hog flesh generate controversy and unease.

Future Prospective and Conclusions

The use of Bioprocesses and Biosystems for Environmental Protection, Microbial Detection, and Prevention in the Food Industry is discussed in this chapter. Although more effort must be done to progress toward a wide sustainable framework, the scientific and technological revolution exemplifies how sustainability & bioprocesses are topics of substantial welfare and are continuously evolving. Since it enables prematurely understandings and errors intervention in production lines at an accelerated pace with authentic specimen tests starting in this era. Nanotechnology has advanced as the foundation of real-time environment and foodstuffs surveying. As another choice, the current pattern uncovers that carbon-based and crossover nanocomposite (GO, GQDs), miniaturization for onsite investigation, and 3D chip engraving are being concentrated on as the business future bearings. This is a outcome of increased automation, data accessibility, and data handling convergence. Big data directs to how the pervasive amount of information has progressed further standard databanks. To investigate, narrative, and construct the data surplus with inferior latency in authentic term, environment and food security monitoring. Adulteration detection, content assurance, Freshness indicators, and foodstuff processing monitoring are some potential uses for biosensors. In the food sector and environment, competent workers frequently conduct routine chemical and microbiological testing. The extraction or pre-treatment of materials makes this research expensive and time-consuming. Biosensors, which offer rapid, non-destructive, and affordable quality control solutions, can address all of these shortcomings. To solve the issues the food business and environment are experiencing, biosensors have the potential to spark an analytical revolution. This chapter gives a general review of the many biosensor types used in the food industry and explores their future possibilities.

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Chapter 12
Application of Biotechnology in the Food Industry

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Introduction

Biotechnology is a vast area of science and has exhibited pertinence in human development for centuries (Sugumaran & Ponnusami, 2017). Biotechnology is extended to various fields, including tissue culture, fermentation, DNA fingerprinting, selective breeding, and recombinant DNA technology (Daddiego et al., 2017; Kamle et al., 2017; Krasznai et al., 2017; Ledoux & Antunes, 2017; Lucarini et al., 2016; Nitschke & Silva, 2016). This technology is also involved in treating various infectious diseases and genetic disorders through the complete analysis of genes/DNA (Calvo-González, 2016; Hamad et al., 2017; Keskin et al., 2004; Lao et al., 2017; Šuster et al., 2017). Food processing, by definition, means to apply various operational methods and technologies in order to convert raw, bulky, and perishable, food materials into sustainable, and palatable food commodities (Swetwiwathana & Visessanguan, 2015). Presently, there is a growing concern about low-cost production and wholesome food products of high value for improving the health of human beings.

The application of biotechnology in the food industry has a consequential effect on the living population. Biotechnology can potentially resolve the need for food and help to avoid mass starvation in the future. It is the field of increasing food productivity, enhancing its nutritional content and organoleptic properties.

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Biotechnology leads to the better utility of food by removing allergens and toxic components. It could also contribute to food security while assisting in promoting sustainable agriculture in developing countries. Globally, consumers are increasingly concerned about food safety and quality. The awareness of replacing chemical additives with natural ones has resulted in an increasing demand for food products enriched with bioactive compounds that would beneficially affect human health (Miguel et al., 2013). Therefore, in the present era, different gluten-free and dietary fiber-enriched food products and food products containing probiotics and oligosaccharides are easily found in the market (Patel & Goyal, 2011).

Lipase acts at the aqueous and a non-aqueous interface. When the water activity is low it synthesizes ester from glycerol and long-chain fatty acids. A "true lipase" splits long-chain fatty acids and emulsified esters of glycerine, e.g., tripalmitin and triolein. The applications of commercial enzymes have been increasing significantly in the last decades, particularly in the food industry (Anishetty & Gowtham, 2017). The biotechnological applications employing living organisms, or derivatives modify products for specific use. Yeast is utilized in fermented products, functional food, and nutraceutical production (Padilla et al., 2015; Rai & Jeyaram, 2017; Rai et al., 2017).

Functional foods by definition, are aliquot of a diet and possess health benefits in excess of their nutritional attributes, whereas nutraceuticals comprise purified components of food that holistically demonstrate health benefits. Genetically modified food (GMF) is also synthesized by using various measures of biotechnology. Modern Biotechnology is also termed transgenic or genetic engineering technology in which Nuclear DNA is altered by inserting a gene that encodes a desired trait and is termed recombinant DNA. The expression of recombinant DNA encodes desired product and when employed to improve food characteristics or yield is defined by the term food biotechnology (Morin, 2008). Modern Biotechnology enhances yield, shelf life, taste, and nutritional values, and also facilitates fermentation and enzymatic processes. In developing countries, biotechnology has the maximum potential to remove malnutrition, hunger, and various diseases in developing countries. Products of modern biotechnology are reasonable at a commercial level and therefore can improve food and agriculture, which would result in an increase in the economic status of farmers as well (Adenle, 2011).

Biotechnology in Food Industries

In food processing industries, biotechnology is profound for the better productivity of food products. The various aspects of biotechnology employed in food processing sectors encompass increasing the food yield, improving the nutritional value of food products, using the fermentation process to produce different products, improving their shelf life, and organoleptic properties, and enhancing food safety (Lokko et al., 2018; Nguyen et al., 2017).

Biotechnology for Better Yield

Transgenesis is done with the aid of biotechnology in which a gene of one organism is manipulated into another of a similar or distinct species to result in the gene expression which is then transferred to the next generation (Song et al., 2017; Zhu et al., 2016). Rats, mice, sheep, cows, rabbits, etc., are some of the examples (Srinivasa & Goswami, 2007). Genetically engineered salmon, a fish, is disease resistant and possesses improved tolerance to environmental stresses (Dunham & Su 2020; Forabosco et al., 2013). The United States Food and Drug Administration approved it as a safe and healthy food for human consumption. Incorporation of extra gene copies that encode κ-casein and bovine β- to female bovine fibroblasts revealed an 8–20% increase in β -casein in milk produced from such animals and an altered κ-casein to total casein ratio (Brophy et al., 2003). In poultry, growth traits have been observed to be associated with polymorphisms in the growth hormone, ghrelin, lambr1, growth hormone receptor, MC3R, IGF-II, MC4R, and TGF-β (Fang et al., 2007; Huang et al., 2007; Jiang et al., 2002; Li et al., 2002; Qiu et al., 2006; Yan et al., 2002). Genetically modified food technology has been adopted for commercial production of GMF and allergenicity tests, digestion, and genetically modified food (GMF) toxicity. Biotechnology has aided scientists in producing GMF with better taste and the seeds eliminated from such food articles resulted in more soluble sugar and enhanced sweet taste (Falk et al., 2002). Biotechnology has modified the pathways of fermentation pathways to add aroma.

Improvement in the Nutritional Value of Foods

With advancing biotechnology, food bio-fortification by recombinant DNA technology and fermentation processes is becoming advantageous in food industries (Cashman & Hayes, 2017). The term Designer food, which was introduced in Japan in 1980, is defined as processed food that contains nutrients resulting in additional benefits of health besides its nutritional status. Cruickshank started the approach of designer eggs, and by making the feed interventions, he observed the modification of fatty acid composition in the yolk of the egg. The designer eggs adorned with omega-3 fatty acids exhibited better polyunsaturated fatty acid stability during the cooking of egg and storage, and high availability of nutrients such as carotenoids, vitamin E, and selenium improved omega-3 and antioxidant content in people consuming eggs (Surai & Sparks, 2001). Several research scientists have developed a wide range of designer eggs, which have been observed to contain omega-3 fatty acids and antioxidants (Sim & Sunwoo, 2002). Raes et al. developed a designer egg supplemented with linoleic acid (Raes et al., 2002). Eggs supplemented with vitamin A and β-carotene have also been developed. By removing the β-LG gene from bovines, allergy to cow milk in children can be minimized (Sabikhi, 2007). Researchers have also recommended that chicken and beef enriched with selenium

can be produced by incorporating organic selenium poultry and farm animal feed (Fisinin et al., 2009). Functional foods are fathoming much importance by playing a significant role in preventing diseases and promoting health benefits (Fisinin et al., 2009).

Biotechnology in Fermentation

In fermentation processes at a commercial level, starter cultures have been utilized to produce different food products of high value (Holzapfel, 2002). Restricting activity of these cultures has been observed due to various substances such as hydrogen peroxide, bacteriocins, diacetyl, and organic acids (Hutkins, 2006). Plasmid transfer, cloning, protoplast fusion, and transduction of the starter cultures have been employed to enhance the anti-cholesterolemic properties, resistance against enteropathogens, and anti-carcinogenic activity of livestock foods. Fermented milk products positively affect the intestines and possess good health benefits (Berni Canani et al., 2017). In fermented dairy and meat product preparation, strains of lactobacillus can be potentially used as probiotics (Pennacchia et al., 2006). Fermented foods are value-added products that are rich in nutrients, prolonged shelf life, are easy to digest, and are more beneficial for the intestinal tract. Thus, biotechnological measurement can be employed to yield enhanced bacteria, yeast, and mold strains, utilized for preparing fermented dairy and meat products.

Biotechnology for Increasing the Shelf Life of Food

The food shelf life is being enhanced by bacterial fermentation. Most fermentation processes involve the sugar conversion by lactic acid bacteria to lactic acid. In the present era, lactobacilli are gaining attention due to bacteriocins production (Collins et al., 2017). These constituents can be used as a natural preservative in the food industry. Lactic acid bacteria and their metabolized products are generally considered safe (GRAS) (Patel & Prajapati, 2013). Bacteriocins can be obtained by harboring specific bacterial cultures in a controlled environment. Nisin has been officially utilized in the food industries and approved worldwide for its utilization (Kaškonienė et al., 2017). The direct addition of nisin to food products, including cheese, flavored milk, canned foods, etc., has also been permitted. A multitude of refrigerated vacuum-packaged processed dairy, fish, meat, and vegetables contain strains of Lactobacillus, Leuconostoc, Brochothrix, Clostridium, and Carnobacterium (Rodríguez et al., 2002). They get multiplied at refrigerated temperature and cause product spoilage. Reduction in Listeria count has been achieved by adding a culture of Lactobacillus sakei in chilled cured pasteurized sliced vacuum-packaged meats and chilled raw ground meat (Devi & Halami, 2011).

Enhancement in Organoleptic Properties of Food

The organoleptic property of food plays a significant role in the fathomable acceptance of food products. Biotechnology has a major role in the evolution of chemical, nutritional, technological, and organoleptic properties (Smaldone et al., 2017). Microbial culture in food production can also enhance the organoleptic properties of food product. A study has estimated that more than 100 commercial chemicals of aroma have been derived by using biotechnological measures (Berger, 2009). The organoleptic attributes of fermented food products are profound, in terms of color, taste, flavor, and aroma (Singh et al., 2012). The Recombinant DNA technology has enhanced efficiency in non-nutritive sweetener production such as aspartame and thaumatin (FAO, 2010). The lactic acid bacteria are used in producing a diverse range of food products. Lactobacillus delbruekii, Lactobacillus helveticus, and Streptococcus thermophilus produce diacetyl compounds that produce flavor and are employed to produce acidophilus milk, yogurt, and high-scalded cheese.

Biotechnology in Food Safety

The European Food Safety Authority has proclaimed that bacteria used in the production of feed carry acquired resistance genes that might jeopardize the living population (EFSA, 2007). Ensuring food safety is important to provide appropriate safeguards for a consumer and encourage trade. Contamination of microorganisms is monitored in the final product and during the production process, sanitation, and cleaning and is one of the important factors in the process of manufacturing in food and biotechnology (Ochoa & Harrington, 2005). Genomics and proteomics technology provide more specific methods for checking microbial contamination of food. Various tools of biotechnology, including PCR (polymerase chain reaction), genetic engineering, amplified fragment length polymorphism, recombinant DNA technology, random amplified polymorphic DNA (RAPD), etc., are being used, and they tend to aid in the authentication of meat and checking its speciation. Development and expansion of new methods for evaluating high-risk pathogens in food products are enormously crucial in the context of food safety (Naveena et al., 2017).

Oligosaccharides

These are the carbohydrates that are the polymers of monosaccharides linked by glycosidic linkages. They have a wide application in the food industry. Oligosaccharides are obtained naturally and chemically or through various biotechnological processes (Pinelo et al., 2009; Villares, 2010). Amongst the various functions, their prebiotic potential is one of the attention-seeking attributes. An

oligosaccharide is prebiotic, not to be absorbed or hydrolyzed in the upper part of the gastrointestinal tract, and therefore undergoes assimilation selectively using a multitude of microorganisms in the colon that promote systemic or luminal benefits. Microorganisms must be safe, multiply and colonize the tract, and be able to survive through the tract (Rioux et al., 2005; Roberfroid & Prebiotics, 2008).

Oligosaccharides like fructo oligosaccharides (FOS) and galacto oligosaccharides (GOS) have been extensively analyzed for their prebiotic benefits. FOS is present in little quantity in natural sources, including sugar beet, asparagus, onion, garlic, wheat, Jerusalem artichoke, banana, honey, tomato, etc. (Sangeetha et al., 2005b). Their large-scale production is also restricted by seasonal conditions from natural resources (Sangeetha et al., 2005a). With the consumption of FOS, various health benefits are associated, including colonic microflora modulation, activation of the immune system, gastrointestinal physiology improvement, facilitation of mineral availability, reduction in serum cholesterol, triglyceride, and phospholipid level, and prevention of colonic carcinogenesis (Charalampopoulos & Rastall, 2012; Lopez et al., 2000; Nemukula et al., 2009). FOS can be employed as a non-carcinogenic and zero-calorie sweetener. 1-Kestose is observed to increase the power of sweetness in comparison to other sc-FOS, and 1-ketose-rich sc-FOS syrups can potentially replace sugar for diabetic patients (Mabel et al., 2008).

Several GOS are considered prebiotics because of their undigestible nature and can be selectively used in the human intestine, thus improving human health (Oliveira et al., 2011; Villamiel et al., 2014). GOS applications are slowly increasing globally because of their significant health benefits. They are present in bakery products, yogurt, beverages, etc. (Venica et al., 2015). GOS potentially stimulates the bifidobacteria and lactobacilli growth in the lumen. GOS can prevent bacterial adherence as they exhibit the property of camouflaging the receptors of host cells in which the adhesion of bacteria occurs (Nauta et al., 2010). They can prevent colon cancer development since they can delay fermentation and reduce genotoxic bacterial enzyme activity associated with this disease (Shoaf et al., 2006). They can stimulate mineral absorption, and their calcium absorption effects have been affirmed. They can alleviate constipation, a common chaos in elders and expecting women. Furthermore, GOS indirectly acts on systemic and mucosal immune activation and protects against allergic manifestations.

Isomalto-oligosaccharides (IMO), including malto-oligosaccharides, are produced from starch. IMOs naturally occur in numerous fermented food products and sugars such as honey, soybean sauce, etc. IMOs exhibit mild taste and are relatively inexpensive. They exhibit relatively low viscosity, less sweetness, and bulking characteristics. They were also utilized as a sugar substitute for diabetics and to prevent dental caries (Zhang et al., 2010) Among the prebiotic oligosaccharides, IMOs are largely employed in food industries. They are widely used as food ingredients (Bharti et al., 2015). IMOs are commonly used for being highly stable, available, and cost-effective (Nguyen & Haltrich, 2013). The benefits of IMO consumption have been evaluated in some studies that investigate health conditions in specific population. IMOs have been observed to stimulate bowel movements and reduce total cholesterol level with an intake of 10 g/day in elders (Meyer, 2015).

Enzymes in Food Processing

Enzymes are being utilized in food production and processing at the industrial level. Food processing industries have used enzymes produced through genetically modified organisms for decades. These enzymes comprise proteases and carbohydrates. To get a higher production of such enzymes in a short period, cloning has been done to the genes. These types of enzymes are used in cheese, and curd making, as well as for food flavoring items. A high amount of such enzymes is utilized in food industries. In the United States, more than 50% of proteases and carbohydrates are used in the food industry. They comprise renin and α-amylase. Some of the enzymes that are genetically modified and used in food industries are mentioned below:

- Catalase in the production of mayonnaise and in removing hydrogen peroxide
- Chymosin as a milk coagulant in the cheese production
- Glucose oxidase in baking to stabilize the dough
- α-amylase in converting starch into maltose and in sweetness baking
- Protease in meat tenderness and in baking and milk products

Enzymes have been used for cheese production and indirectly through yeasts (Schmid et al., 2001). Pectinases, containing various enzyme activities, have been used in fruit juice manufacturing that aid in clarifying juice. Microbial enzymes are majorly used in starch industries. The starch hydrolysis has been substituted by glucoamylases and α -amylases, converting approximately 95% of starch to produce glucose. Xylanase and cellulase enhance the juice extraction from the pulp in addition to pectinase. Pectinase and amylase are used in the juice clarification. The various applications of different enzymes in the food processing sectors is listed in Tables 12.1 and 12.2 (Shakuntala et al., 2009).

Microbial enzymes are widely employed in the food processing industry. The worldwide β-galactosidase production has been enumerated at approximately 5.749 million MT per year. It helps in removing lactose from milk and producing galactosylated products (Husain, 2010). Research studies have shown that nearly 70% of the worldwide population of different age groups is not able to digest lactose. However, a low intake of dairy products besides being rich in calcium leads to a higher risk of fractures, such as osteoporosis. Lactose hydrolysis in milk before consumption has been observed to aid monosaccharide absorption. New technologies have been developed to produce dairy products that are lactose-free, including lactose hydrolysis. Thus, β-galactosidase hydrolytic activity in the food industries has fathomed global acceptance in the decades for the lactose reduction in milk trans-glycosylation reactions for GOS synthesis in a few past years (Oliveira et al., 2011; Park & Oh, 2010).

Hydrolysis of lactose has acquired much significance from technological, clinical, and environmental perspectives. Disaccharide hydrolysis increases the solubility of lactose and the product sweetness due to galactose and glucose thereby using a greater quantity of constituents of whey. β-Galactosidases can be produced from yeasts, bacteria, and filamentous fungi (Oliveira et al., 2011). β-Galactosidase

Table 12.1 Enzymes used in food processing industries

Industry	Enzyme
Butter oil and butter	Lipase, glucose oxidase, catalase
Cheese	Lipase, proteinases, rennet
Animal feed	Glucanase, amylase, pentosanases, glucoamylases, cellulases, xylanases, proteinases, phytases
Biscuits	Amylases, hemicellulases, cellulases, pentosanases, proteinases
Bread	Amylases, cellulases, amyloglucosidases, glucanases, glucose oxidase, hemicellulases, proteinases, pentosanases, lipases
Brewing	Decarboxylase, acetolactase, amyloglucosidase, amylases, glucanase, cellulase, lipase, proteinase, pentosanase, and xylanase
Coffee	Galactomannanase, cellulase, pectinase, hemicellulases
Confectionery	Amylase, invertase, pectinase, proteinase
Egg processing	Proteinase, lipase phospholipase, catalase, glucose oxidase
Dairy products	Lactase, sulphydryl oxidase, proteinase, lysozyme, lactoperoxidase, peroxidase, and catalase
Flavor	Glucanase, proteinase, peptidase, lipases, esterase, amylase
Fat	Esterase, lipases, glucose oxidase
Fish	Proteinase
Cloudy juices and fruits	Proteinase, amylases pectinases, cellulases
Fruit extract	Anthocyanase
Fruit and vegetable processing	Cellulases, macerating enzymes, pectinases
Tea	Cellulase, glucanase, pectinase, tannase
Protein	Glucanase, amylase, cellulase, pectinase, protease, hemicellulase
Starch	Amylase, cellulase, hemicellulase, isomerase, glucanase, lipase, pectinases, proteases, phospholipase

Shakuntala et al. (2009)

Table 12.2 Enzymes in food as additives

Enzyme	Application
α-amylase	Ethanol fermentation, starch syrups, animal feed
β-amylase	Maltose syrup, brewing
Cellulase	Animal feed
β-glucanase	Brewing
β-glucosidase	Transforming isoflavone phytoestrogens in soymilk
Dextranase	Dextran hydrolysis
α-galactosidase	Increased yield of sucrose; potentially used in the sugar beet industry
Invertase	Manufacturing inverted syrup from cane sugar
Lactase	Eliminating lactose from milk and milk products
Pectinase	Fruit processing
Protease	Baking goods, brewing, protein processing, meat tenderization, distilled spirits

Shakuntala et al. (2009)

exhibits various functional characteristics which could be further enhanced by isolating microorganism strains, yielding immobilized enzymes that are receptive to chemical mutagenesis, improved secretion of enzymes, and expression of genes by recombinant DNA techniques are continually increasing in the present era. Recombinant DNA technology is currently used in expressing and optimizing β-galactosidase production from the most diverse sources. Moreover, β-galactosidases enriched with properties such as higher yield of product, reduced inhibition of product, etc. could also be produced by using protein engineering techniques (Gosling et al., 2010).

Fungi in Processed Food

Fungi comprise an impartial part of food, both as animal and human food. Single Cell Protein (SCP) is termed as a group of various microbial products of fermentation and can be employed in the fermentation of several waste effluents, such as wood, straws, food, and their processing wastes, alcohol, and residues of human and animal excreta. They are commonly produced as diluted solutions comprising less than 5% of solids, that undergo precipitation, filtration, centrifugation, and coagulation. Material stabilization for storage water removal is a mandatory step. SCP must contain about 10% moisture content or need to be acidified and condensed to keep spoilage at bay. It should be fed immediately after its production. Nevertheless, yeasts have better utility compared to other types of fungi (Shakuntala et al., 2009).

Fungi: An Alternative to SCP

Mycoprotein, namely Quorn is the most notable processed fungal food. The product is available as high-protein SCP flour. It has been developed as a substitute for meat on the basis of its organoleptic properties. In a larger air-lift fermenter the mycelium is continuously cultured. It has a filamentous structure that tends to induce the fibrous attribute of meat associated with the fungal nutritive value. The product is a less-calorie, low-fat healthy food and is also free from cholesterol (Shakuntala et al., 2009). The various uses of processed fungal food are mentioned as follows.

Application in Fermentation-Based Food Industries

Macro fungi cultivation has flourished for a few years, but the fresh mycelia used as food have still not gained popularity globally. Furthermore, the function of such organisms, particularly yeast in the production and processing of food is indispensable. Fungal cell factories are greatly used in bread-making and brewing industries as they secrete a wider range of enzymes into the culture medium (Shakuntala et al., 2009).

Cheese and Bakery Products

Bakeries particularly comprise wheat flour mixed with water, sugar, and salt, using yeast as a leavening agent at the incubation temperature of 25 °C. The yeast aids in the fermentation of sugar and produces alcohol and carbon dioxide. The liberated gas forms bubbles by the extension of gluten in the flour. During backing evaporation of alcohol occurs. Bread flavour and texture are estimated by various factors, such as amount of gluten in the flour, the length of leavening, the constituents of grain, and the temperature. The appearance of mycelium is a part of moldy cheese favorites amongst gourmets in the production of cheese. Camembert and Roquefort also termed blue cheese are synthesised by two species of Penicillium, P. roqueforti in Roquefort cheese and P. camemberti in Camembert cheese (Shakuntala et al., 2009).

Other Food Products

A multitude of research has been done on various fermented products of food that lead to the establishment of the fungus identification included in the process, such as shoyu, miso, tempeh, and tofu. In spite of this, various microorganisms, such as bacteria used in the major fermented food products are unidentified. In Western culture, yeasts usually play a role in the fermentation, whereas the East has used a multitude of mycelial fungi. Among the various Asian food products soya sauce is one of the most familiar. Shoyu is a flavor enhancer. Tempeh is considered to have its origin in Indonesia and is prepared from the fermented legume seeds using Rhizopus oligosporus. Miso, a Japanese word given to fermented paste of soybean is consumed as a soup base or used as a flavour enhancer. During its fermentation, rice is washed, polished, steamed, and inoculated with Aspergillus oryzae, which results in the formation of rice koji which is further inoculated by bacteria and yeast and proceeds to fermentation (Shakuntala et al., 2009).

Fungi in a Regulated Diet

The fungal food consumption has increased globally in recent years with increase in public demand for health concerns. Vegetarians have recoursed to consuming freshly cooked mushrooms, beverages, and dietary supplements that are of fungal origin (Shakuntala et al., 2009).

Fruiting Body Utilization

The fruiting body of mushrooms has been consumed as fresh or in processed form and as a delicacy. Fungi can be technically produced through fermentation, media preparation, inoculation, and incubation. The culture media tends to be available in the form of substrates from sources of low value, including agricultural and industrial waste, and are transformed into food products of high value. Therefore, the utilization of fungi is economically important as well as eco-friendly. A multitude of edible mushroom species exist wildly approximately 19 species are widely used as food and nearly 8–10 are cultivated on a regular basis. The most common edible species, like Agaricus bisporus, when small are sold as button or portobello mushrooms when larger, and used in soups, and various dishes. Most of the fungi of Asian origin are being cultivated at a commercial scale and have been popularised in the West. They are also available in markets, such as grocery stores, which include oyster mushrooms, straw mushrooms, enokitake, and shiitakes. There are various other fungi like milk mushrooms, morels, truffles, porcini mushrooms, and black trumpets also known as king boletes, which are costly. The most common edible macrofungi are given in Table 12.3, exhibiting their medicinal and nutritional attributes, and can be utilized as a substitute for non vegetarion protein sources (Shakuntala et al., 2009).

Biotechnological Applications of Yeast in Food Industries

Yeasts highly participate in producing various types of nutraceuticals and functional foods (Padilla et al., 2015; Rai et al., 2017). Functional foods, part of a normal diet are conventional commodities and exhibit various health benefits in excess of their nutritional attributes. Nutraceuticals comprise purified components of food that are proven to evince health benefits. Yeasts enhance bioactive components in fermented food by producing enzymes and metabolites, or they act synergistically with other classes of microorganisms to improve their functional characteristics (Rai et al., 2016). Yeasts are widely applied in functional food industries, such as

- Living cells can be utilized as probiotics
- Cell wall components, such as β-glucan exhibiting nutraceutical value
- Extracellular fractions that are secreted consist of folate, carotenoids, γ-amino butyric acid
- Specific enzyme producers are the key players in the biotransformation of food metabolites that result in producing nutraceuticals of high value (Padilla et al., 2015; Rai et al., 2016)

Table 12.3 Common edible macrofungi with nutritional and medicinal properties

Fungi	Nutritional properties	Medicinal properties	References
Straw mushroom	Source of antioxidants because of the presence of β-carotene in high quantity	Contains FIP-Vvo, that stimulates TH1 and TH2-specific cytokine	Cheung et al. (2003)
Winter mushroom	Contains mannofucogalactan which is a hetero galactan derivative of Flammulina that possesses nutritional attributes	Causes production of antibody by modulating the differentiation and function of TH-cell	Carbonero et al. (2008)
Oyster mushroom	Exhibits flavor and aroma; rich in carbohydrates, protein, fiber, vitamins, and minerals	Exhibiting antiviral hematological, antibiotic, antitumor, immunomodulation, and antibacterial attributes	Cohen et al. (2002)
Truffle	It has a tempting aroma and taste and is economically the desired delicacy	Therapeutic having anti-cholesterolaemic, anti-carcinogenic, viral, and prophylactic properties regarding to hypertension and coronary heart disease	Carbonero et al. (2008)
Reishi	It is employed in dietary preparation. Protein includes about 7.29% of dry weight. Metals and glucose account for about 10.21% and 11% of dry mass respectively, including Mg, K, Ca and Ge, being in major quantity	GLIS stimulates the activation of B lymphocyte, proliferation, differentiation, and immunoglobulin production	Bao et al. (2002)
The common morel	Morels are a feature of many cuisines, such as provencal	Antioxidant and scavenging activity, reducing power, and chelating effect; also comprises galactomannan inducing macrophage activity	Duncan et al. (2002)
The black morel	Rich in Vitamin D2	It is believed to cure the common cold, tuberculosis and high blood pressure	Mattila et al. (2000)
The half-free morel	The spongy texture of young morels is used to make delicious dishes	The ethanolic extract of Morchella has 85% antioxidant properties	Carbonero et al. (2008)

Shakuntala et al. (2009)

Baker's Yeast

The baker's yeast production is largely used for various purposes in food processing. It is a Saccharomyces cerevisiae strain that is selectively used to produce abundant gas of desired flavor. The organisms and bread dough are mixed to vigorously initiate the fermentation of sugar. The liberation of carbon dioxide gas during fermentation leads to the leavening of the dough (Shakuntala et al., 2009).

Yeasts Used as Probiotics

The various qualities needed for a microorganism to be considered probiotics include growth at high pH, capacity to withstand bile juice, hydrophobicity surface of the cell, and auto-aggregation (Fadda et al., 2017). Various research studies have been done on probiotics in the bacterial system, however, yeast has evinced its capability as a probiotic (Saber et al., 2017a, b). Yeasts are advantageous over bacteria since they are nearly 10 times bigger than bacteria and tend to resist antibiotics during antibiotic treatment. Yeast isolated from fermented food products has also been analyzed for its ability in the assimilation of cholesterol and its probiotic effect. A probiotic yeast, K. marxianus CIDCA 8154 has been observed to reduce oxidative stress and inhold an anti-inflammatory effect (Romanin et al., 2015).

Constituent of Yeast Cell Wall as the Ingredient of Functional Food

Yeast cell is a source of fiber and β-glucan that enhances immunity, reduces blood cholesterol level, and exhibit anti-inflammatory effect (Vieira et al., 2016). The yeast cell wall composition changes accordingly with various genera and is dependent on various conditions of growth, affecting the functional characteristics of its polysaccharide (Galinari et al., 2017; Jaehrig et al., 2008). The composition of cell wall of S. cerevisiae has been observed to comprise β (1 → 3)-D-glucan (50–55%), β (1 → 6)-D-glucan (5–10%), mannoprotein complex (35–40%) along with chitin (2%) (Kwiatkowski, 2009). Yeast beta-glucans, also called Saccharomyces β-glucans have been approved by EFSA for their role as novel food ingredients with available range between 50 and 200 mg (EFSA, 2011). In fermented foods, constituents of yeast cell wall are a potentially produce bioactive molecules that impart functional attributes to the product.

Nutraceutical Production from Yeast

Yeast is well known for nutraceutical production that prove to be an important part of the food industry, including folate, carotenoids, and γ-aminobutyric acid (GABA) (Chen et al., 2016; Greppi et al., 2017; Han & Lee, 2017). Products fermented by yeasts have been observed to comprise various biologically active metabolites that improve the product functionality. Yeast has been observed to produce GABA by glutamate decarboxylase (Han et al., 2016; Han & Lee, 2017). Folate comes under the category of essential cofactors in various biochemical reactions, and its poor availability in the diet has become one of the concerns worldwide (Greppi et al., 2017; Korhola et al., 2014). Yeasts that are used for producing functional foods have

resulted in fermented foods rich in folate (Hjortmo et al., 2008a, b; Kariluoto et al., 2006). Carotenoids are naturally pigmented compounds and play a crucial role in the food industries as they prevent oxidative stress-related diseases (Chen et al., 2016; Mannazzu et al., 2015). Carotenoids produced by yeast include β-carotene, astaxanthin, γ-carotene, torularhodin and torulene (Mannazzu et al., 2015; Moline et al., 2012). A non-proteinaceous thiol peptide, glutathione is able to diminish the negative effects of oxygen radicals, which makes it one of the phenomenal components for the application of nutraceuticals (Liang et al., 2009; Musatti et al., 2013). The widely studied yeasts, S. cerevisiae and Candida utilise have been observed to produce glutathione (Liang et al., 2009; Musatti et al., 2013). In fermented products, the inclusion of strains of yeast to produce these metabolites tends to have a positive influence on the consumption level (Rai et al., 2018).

Yeast in Biotransformation to Produce High-value Nutraceuticals

During fermentation processes, a wide range of biochemical changes occur due to the production of enzymes depending on substrate-specific nature (Rai et al., 2017). These changes result in the hydrolysis of the complex substrate to a simpler one and the transformation of the biomolecule into its active state. The output depends on the microorganism strains used in the process of fermentation and the composition of the substrate (Rai et al., 2017). Food components and yeast interaction result in the production of various types of metabolites that exhibit specific benefits on health depending on the biochemical composition of the product. Yeast-fermented food metabolites have been reported to possess free polyphenols, bioactive oligosaccharides, and biologically active peptides (Rai & Jeyaram, 2017). In the production of functional food, the yeast association with filamentous fungi positively affects various fermented products and bioprocesses important at the industrial level for nutraceutical production (Feng et al., 2007). Yeast has proven to be an integral part of fermentation associated with filamentous fungi for producing highly pure oligosaccharides exhibiting prebiotic attributes (Guerrero et al., 2014; Nobre et al., 2018; Sheu et al., 2013). Yeast associated with filamentous fungi is a promising opportunity for the development of functional foods (Rai et al., 2018).

Applications of Lipase in Food Industries

Lipase is a group of hydrolases that takes part in triglyceride hydrolysis to yield glycerol and free fatty acids. Lipase is employed in two different ways. It is used as a biocatalyst in the making of food ingredients and is employed in the development of fine chemicals. Lipase is commonly used in food processing, oil and fat processing, textile, leather, degreasing formulations, detergents, pulp, paper processing,

Table 12.4 Lipase enzymes in food processing industries

Industry	Role	Application
Dairy	Fat hydrolysis, butter fat modification, ripening of cheese	Flavoring agent development in milk, butter, and cheese
Bakery	Enhancement of aroma and flavour	Increment in shelf life
Food dressing	Quality enhancement	Mayonnaise and dressings
Dietary foods	Transesterification	Dietary products
Fish and meat	Flavour enhancement	Fat removal of fish and meat
Oil and fat	Hydrolysis and transesterification	Fatty acids, cocoa butter, margarine, mono, and diglycerides
Processing of tea	Lipid breakdown and flavour enhancement	Black tea

Joo et al. (2002)

production of pharmaceuticals, synthesis of fine chemicals, cosmetics, etc. (Houde et al., 2004). The utilization of lipase in food industries is to modify and hydrolyze biomaterials. Most commercially produced lipase enzymes are used for flavor enhancement in milk products and other food processing, such as vegetables, meat, fruit, baked foods, etc. A diverse role of Lipase enzymes in food processing industries is shown in Table 12.4 below.

Lipase in the Dairy Industry

Lipase is commonly used in the dairy industry for the hydrolysis of milk fat. In the dairy industries, lipase enzyme is used to alter fatty acid chain length and in the flavor enhancement of various types of cheese. The other applications of lipase in the dairy industry include increasing cheese ripening and fat, butter, and cream lipolysis (Sharma et al., 2001). The free fatty acids produced by lipase activity on milk fat produce soft cheese exhibiting specific flavor attributes. Pre-gastric tissues and pancreatic glands of young ruminants, including calf and lamb are traditional sources of lipases used in the enhancement of cheese flavor (Aravindan et al., 2007). Gastric lipase has been used in accelerating the development of flavor and chees ripening, e.g., provolone, cheddar cheese, and ras cheese.

The addition of lipase has been reported to enhance the rate of liberation of fatty acid, which tends to accelerate flavor development. The research study has shown that the supplementation of calf lipase and the increase in the ripening temperature in the range 7–53 °C resulted in an increase in the liberation of fatty acids. However, the lipase continued to remain in its active form after ripening which could cause the development of a strong rancid flavor. The addition of a high concentration level of lipase enzyme during ripening of cheddar cheese tends to result in increased enzymatic reactions that could deteriorate the desirable attributes and thereby decrease the yield. The liposome technology adaptation to accelerate the ripening of cheese has been observed to reduce bitterness and yield losses.

Lipase in the Bakery Industries

In bakery industries, a high focus on lipase enzymes is of great concern. Various research findings have suggested that lipase enzyme can be used in substitution to traditional emulsifiers it potentially degrades wheat lipids in order to produce emulsifying lipids. Lipase was mostly used for flavor improvement in bakery by releasing fatty acid short chains through esterification. In addition to the enhancement of flavor it has also been observed to modify the natural lipids in flour to strengthen the dough and prolong the shelf life of bakery products (Sachan & Singh, 2015). In A. oryzae, an artificially imparted lipase has been used as a processing succour in baking industries. Lipase, including all the hydrolytic enzymes, has been found to effectively reduce the initial firmness and increase the specific volume of slices of bread (Keskin et al., 2004). The increment in butter flavor for baked goods has been produced by butterfat hydrolysis with suitable lipase.

Meat and Fish Processing and Food Dressing

In meat and fish processing, lipase is utilized for removing fat and adding flavor. Lipase is found in fats and oils, which leads to fat breakdown into free fatty acids and glycerol. Lipase is also present in meat fat, eggs, fish, cereals, and milk. Lipase is widely used in whipping and mayonnaise dressing for texture and quality improvement (Sachan & Singh, 2015).

Lipases in Tea Processing

Lipase is extensively used in the processing of tea. The black tea quality largely depends on, enzymatic fermentation, dehydration, and mechanical braking. During black tea processing, the enzymic hydrolysis of lipids of the membrane commences volatile product formation with distinctive flavor attributes (Verma et al., 2012).

Lipase in Oil and Fat Processing

Oil and fats are important food components and their modification is one of the important areas in the food processing industries that require green and economic technologies. Lipases play a key role in modifying lipid properties by changing the fatty acid chain location in the glyceride and substituting them with other ones. Esterification and interesterification are being employed for obtaining products of high value through the lipolytic conversion of fats and oils (Rai et al., 2018). A research study has made an immobilized lipase membrane reactor for the hydrolysis

of fat and oil, produced products requiring minimized downstream processing and reduced the total cost of processing. The removal of phospholipids in vegetable oils, known as de-gumming, has also been developed as an eco-friendly process (Clausen, 2001). Triacylglycerol lipase generated from genetically modified A. oryzae has been used for oil de-gumming and to enhance the emulsifying properties. A new process for lipase immobilization based on silica granulation has been observed to simplify the process and reduce the processing cost. These methods have been widely applied for oil and fat production free from trans-fatty acids (Christensen et al., 2001).

Lipase as a Biosensor in the Food Industry

Immobilized lipase is effectively used as a sensor to quantitatively determine triacylglycerol as they are fast, accurate, and cost-efficient. The application of such lipase is important in food industries. The primary use of lipase as a biosensor is liberating glycerol from the triacylglycerol analytically and quantifying the released glycerol by an enzymatic or chemical method. An experimental study has developed a method for determining organophosphorus pesticides with a surface acoustic wave impedance sensor by lipase hydrolysis. Lipase immobilization is done on oxygen electrodes combined with glucose oxidase, which is thereby used as a lipid biosensor and tends to be used in determining cholesterol levels in blood and triglycerides (Hasan et al., 2006).

Dietetics

With the increase in risks involved in high intake of fat, an increasing demand for low-calorie fats and substitutes of fat is highly considerable, but should not be vulnerable to high ranges of temperature. The less caloric fats and substitutes of fats do not possess natural fatty acids but may be in line with the function and chemistry of natural fats resulting in the formation of such products that are deficient in essential fatty acids (EFA). The analysis of the tri glycerols has shown an increasing preference for the action of lipase for the primary positions in comparison to the secondary positions. The targeted tri glycerols should be useful both in the formulation of food products for infants and in applications of parental nutrition. The utilization of papain of a good quality enriched with lipase ensures fathomable approval of such products. Lipids containing oleic, palmitic, linoleic, and stearic acid, similar to human milk fat, have been produced by enzymic hydrolysis between fatty acids, stearic acid, tripalmitin, and hazelnut oil. For both stearic and oleic acids, the level of incorporation is increased with the time of reaction. The structured lipids produced have been observed to be used potentially in infant formulae (Sahin et al., 2005). It has also been stated that industry and academia collaboration would hasten the enzymatic processes at as successful commercial level (Undurraga et al., 2001).

Miscellaneous

Lipase enzymes have been usually utilized in producing various products, starting from fruit juices to fermented vegetables. Lipase facilitates fat removal from fish and meat products (Sharma et al., 2001). It has been reported that lipase catalyzes and synthesizes sugar fatty acid esters. An experimental finding suggests that the supplementation of lipase in noodles has resulted in significant and soft textural attributes in noodles even though they have relatively low concentrations of acylglycerols in the formulations (Undurraga et al., 2001). In confectionery processing, lipase has been used in producing fat of high concentrations of 1,3 stearoyl-2-monoolein which could be also used as a substitute for shea stearine in the synthesis of equivalents of cocoa butter. Fats inhibiting the formation of bloom in chocolate products have also been synthesized by these types of enzyme esterification mechanisms (Macrae, 2000). C. rugosa lipases are widely used in the flavor and food industry, in single-cell protein and ice cream production, in the biocatalytic resolution of pharmaceuticals, in esters of carbohydrates, and in amino acid derivatives that are not obtained conventionally.

Immobilized lipase produced from C. antarctica has been used in the esterification of bioactive compounds along with fatty acids. Vitamins and secondary metabolites like kojic acid derived from microorganisms and plants can be acylated to form such products that are beneficial in the cosmetic, pharmaceutical, and food industries. Regioselective modification of polyfunctional organic compounds has been proved as an extended area of the application of lipase. The enzyme is also conjugated with a mixture of microbes for treating effluents enriched with fat derived from ice cream. It might also be used in the waste processing from the food industry.

Conclusion

The applications of biotechnology in the production and processing of food encompass a very large and diverse field. Modern biotechnology is applied in the improvement of food taste, and yield, increasing nutritive values, and shelf life. It is also applied in fermentation and enzymic processes. Therefore, biotechnology can be employed for human health benefits, and eradicate malnutrition, and health ailments in developing countries. The β-galactosidase emerges as more promising in fruit juice preparation and breakdown of plant polysaccharides containing galactose and other hexoses. Genetically modified food technology is amongst the advanced technologies of the present era that would potentially combat hunger, malnutrition, and poverty. However, genetically modified food is opposed by a multitude of people. Various seminars should be conducted to make people aware of the potential pros and cons. Biotechnology should be introduced as an individual subject at the high school level to educate students about its benefits. It can potentially help in

combating many nutrition-related problems in developing countries. Various bioprocesses have been formed involving yeast as a single or a mixed starter along with filamentous fungi and lactic acid bacteria for nutraceutical production. With an increasing focus on yeast participation in the synthesis of nutraceuticals, the selection of potential yeast strains for the enhancement of the functional characteristics of the product has become an integral approach. Recent measures of biotechnology have also been employed to result in recombinant yeast production with enhanced characteristics for producing nutraceuticals. The potential use of lipase enzyme in the food industry demands to development of cost-efficient technologies for high production, scale-up, and purification of this potential enzyme. A myriad of hydrolytic applications, like the flavor development in butter, margarine, cheese, and milk chocolates, is a desirable area of the enzyme lipase. However, the new applications of lipases are yet to be examined in food industries. The lipase properties have been enhanced by genetic engineering to extend its applications in unfavourable conditions. A variety of changes in enzyme immobilization plays a key role in applying lipase as a biocatalyst in food processing and technology.

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Chapter 13
Application of Microbial Enzymes in Food Industry

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Introduction

The utilization of enzymes in food processing is practiced since ancient times. Technology has enabled the development of new enzymes with a broad range of application and specificity (Raveendran et al., 2018). Moreover stable to plant and animal derived enzymes make them appropriate for food industry. They can modified and optimized as per the product need (Gupta et al., 2017). The enzymes derived from microorganism even used to enhance the flavour and texture eventually reduce the coast of outsourcing. Microbial Enzymes applications known across the world in the field of medicine, energy sector and agriculture in recent trends these enzymes are getting attention to researchers due to ecofriendly nature (Choi et al., 2015).

The global market for microbial enzymes was estimated to be worth roughly $4.2 billion USD in 2014, and by 2020, it is anticipated to grow by 7% (Abada, 2019). Consumers choose enzymes over chemical food processing aids because they are seen as natural, non-toxic food components that come from plants, animals, or microorganisms. Although the outlined criteria are far from universal, regulatory bodies evaluate the safety of these industrially manufactured enzymes due to the specific nature of their usage in the food business, which have evident implications for public health. Man has relied on microbes and enzymes to produce food for thousands of years. Common uses for enzymes over many years include the manufacturing of beer, bread and wine.

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Traditional Uses of Enzymes Derived from Microorganism

Enzymes derived from microorganism, have been used in the food processing for a variety of applications, including improving the texture, flavor, and nutritional properties of food products. Few of the traditional uses of microbial enzymes in the food industry include cheese production, brewing, and baking. Cheese production is one of the oldest and most well-known use of microbial enzymes in the food industry. Enzymes such as rennet, which is derived from the stomachs of young ruminants, are used to coagulate milk proteins and form curds, which are then processed into cheese (Harboe et al., 2010). Microbial enzymes such as chymosin, which is produced by the fungus Aspergillus niger, have been developed as alternatives to animal-derived rennet and are now widely used in the cheese industry (Olempska-Beer et al., 2006). Moreover, an important application of enzymes in food industry is brewing. Enzymes such as amylases, which are produced by bacteria and fungi, are used to break down starch into simple sugars during the malting process. This is essential for the production of beer, as yeast requires simple sugars to ferment and produce alcohol (Gurung et al., 2013). Baking is a third traditional use of microbial enzymes in the food industry. Enzymes such as alpha-amylase and protease, application in enhancing the texture and shelf life of bread by breaking down complex carbohydrates and proteins (Dahiya et al., 2020). In addition to these traditional uses, microbial enzymes are also being developed for a range of new applications in the food industry. For example, enzymes such as transglutaminase, which is produced by bacteria, are being used to improve the texture and appearance of meat products (Kieliszek & Misiewicz, 2014). Overall, microbial enzymes have played an important role in the food industry for centuries and continue to be an essential tool for food scientists and manufacturers. As new enzymes are discovered and developed, they offer the potential to improve the quality and sustainability of food products.

Role of Enzyme Derived from Microorganism in Food Biotechnology

Genetic alteration and modification of DNA technology has made it possible for industrial microbes to manufacture enzymes that were initially taken from pathogenic or toxin-producing microorganisms are difficult to culture or even uncultivable. The use of different microbial enzymes in the food processing sector is increasing at an alarming rate. Furthermore Extremophiles are the source of a growing number of these enzymes, however the productivity and yield enhanced, by gene copies and sequences (Dalmaso et al., 2015; Neifar et al., 2015). As a result, a thorough understanding of the metabolism of two important industrial-grade microorganisms, *Aspergilius niger* and *Bacillus subtilis*, makes it possible to produce large quantities of enzymes in a way that is both rational and economical. Enzymes

with increased activity, selectivity, or stability have been designed and produced in large part thanks to advances in protein engineering (Singh et al., 2013). However the solid state fermentation (SSF) has become more important now days, submerged fermentation has historically been the primary method for producing enzymes (Thomas et al., 2013). Industrial bioprocess needs microbial enzymes and a wide spread industrial commercial applications. It is estimated around 500 products manufactured from enzymes. Due to diverse nature of microorganisms, industrial enzymes demand increased as less harmful to environment due to its manufacturing process (Patel et al., 2016).

Microbial enzymes play a crucial role in food biotechnology. These enzymes are used in a wide range of food processing and production applications. Here are some of the ways in which microbial enzymes are used in food biotechnology. Microbial enzymes are used in the production of cheese. Rennet, which is derived from the stomach lining of young ruminants, is traditionally used to curdle milk during cheese production. However, microbial enzymes like chymosin and pepsin can also be used as an alternative (Ogel, 2018). Microbial enzymes are used in the production of bread and other baked goods. Amylase, protease, and lipase are commonly used enzymes that help break down starches, proteins, and fats, respectively, to improve the texture and flavor of baked goods. Microbial enzymes are used in the production of beer and other alcoholic beverages. Enzymes like amylase and maltase help convert starches into sugars that can be fermented by yeast to produce alcohol. Microbial enzymes are used in meat processing to tenderize meat and improve its flavor. Proteases like papain and bromelain are commonly used enzymes in meat processing (Bekhit et al., 2014). Microbial enzymes like pectinase are used in the production of fruit juices to break down pectin, a complex carbohydrate that causes cloudiness and viscosity in juice (Danalache et al., 2018). Overall, microbial enzymes are essential in food biotechnology as they help improve the quality, texture, and flavor of food products, while also making the production process more efficient and cost-effective.

Applications of Different Microbial Enzymes in the Food Industry

Enzymes helps in reducing the energy required to catalyse the reaction, In living organisms. However, enzymes in plants and animals are present in small quantities and cannot be used for industrial applications. Microbial enzymes, on the other hand, offer many advantages, including easy handling, rapid multiplication, genetic manipulation, high production yield, and cost-effectiveness. They are eco-friendly, stable, and can convert hazardous compounds into useful products, making them ideal for use in several industries. Microbial enzymes are well-known biocatalysts for producing various products from a wide range of substrates. Therefore, their production at an industrial scale under varied physical and chemical conditions is

essential. Microbial enzymes produced by microorganisms that act as catalysts to accelerate various biochemical reactions in food. These enzymes are widely used in the food industry for various applications, including fermentation, baking, brewing, cheese making, meat processing, and many more (Singh et al., 2016). There are several types of microbial enzymes, each with a unique role in the food industry. Here are some of the most commonly used types of microbial enzymes and their roles:

Proteases

Enzymes have become highly significant in global industry due to their ability to act as biocatalysts and decreased the minimum energy to catalyse reactions. Lactic acid bacteria are a well-studied a set of microorganisms, and their proteolytic system is particularly important for the utilization of casein and supply of essential amino acids.

The proteolytic system also plays a role in regulate the grade of polypeptides and regulating protein levels. These functions are important for the sensorial characterises of fermented milk products (Kieliszek et al., 2021). Proteolytic enzymes found in microbial cultures are essential in the dairy industry for producing cheese, yogurt, kefir, and other fermented dairy products. These enzymes not only break down proteins but also coagulate milk proteins during the cheese-making process. The resulting protein hydrolysates can be used to make easily digestible dairy products for sick individuals and children. Exogenous proteolytic enzymes are crucial for cheese production (Abada, 2018; Ozturkoglu-Budak et al., 2016). In meat industry proteases break down muscle protein macromolecules, disulfide bonds, and increase the reactivity of certain chemical groups in meat. This leads to the hydrolysis of sarcoplasmic proteins and partial hydrolysis of myofibrillar and connective tissue proteins. The result is tenderized meat, increased hydration, and improved protein digestibility. The content of soluble amino acids and small peptides increases, improving taste and texture while shortening maturation time and protecting against unfavorable microorganisms (Ahmad et al., 2020). Proteolytic enzymes enhance the flavor, texture, aroma, and color of meat products, improving sausage quality. The addition of proteases to meat has been found to have various positive effects, one of which is the ability to delay lipid oxidation. Lipid oxidation is a complex chemical reaction that occurs in the presence of oxygen, resulting in the degradation of fats in meat products. This process can lead to the formation of undesirable flavors, odors, and colors, as well as the production of harmful compounds that are detrimental to human health. By delaying lipid oxidation, proteases can help to preserve the quality and safety of meat products (Lorenzo et al., 2018).

Amylases

Amylases are capable of breaking down starch into reducing sugars. Microbial amylases could be derived from bacteria, fungi, and yeast. Amylases able to catalyse carbohydrates especially starch to simple units. Microbial amylases are widely used in the food processing for their ability to enhance texture, flavour, as well as nutritional value of various food products. They have application in manufacturing of glucose syrup, sweeteners, and alcoholic drink. The sources of microbial amylases include Bacillus, Aspergillus, and Rhizopus species, among others. These enzymes are more selective compare to other sources of amylases due to their high yield, stability, and ease of production. Amylases are widely used food product manufacturing, and they represent 25% of the global enzyme sell (John, 2017).

Pectinases

These enzymes are derived from microorganisms, including bacteria, yeasts, and actinomycetes. However, filamentous fungi are known to be particularly effective in producing pectinases. These enzymes are responsible for breaking down pectin, a complex carbohydrate found in plant cell walls, and to enhance the functional properties of food, juices and extracts. Fungal pectinases are considered to be among the most effective in terms of their ability to break down pectin, and they are often used in large-scale production processes (de Souza & Kawaguti, 2021). The use of pectinolytic enzymes is an important part of food processing, as it allows manufacturers to create a wide range of products with improved texture, flavor, and consistency (Jahan et al., 2017). Pectinases are used to clarify fruit juices by breaking down the pectin molecules that can cause cloudiness in the juice. This process helps to produce clear and smooth juice (Grassin & Coutel, 2010). These are used in the wine industry to improve the extraction of juice from grapes and to clarify the wine during the production process (Tapre & Jain, 2014). These enzymes are also used to soften fruit and to facilitate the extraction of juice during fruit processing. They are also used in the production of jams, jellies, and other fruit-based products. Pectinases are used to improve the quality of baked goods such as bread and cakes. They help to improve the texture and increase the volume of the products (Ozatay, 2020). Overall, pectinases are helpful to maintain the quality, texture, and consistency of various food products.

Cellulases

Microbial cellulases are derived from microorganism are responsible for breaking down cellulose, to glucose and fructose. Cellulases are widely used in the food industry for their ability to improve the texture and flavour of various products. One

of the most important applications of microbial cellulases in the food industry is in the production of fruit juices. Cellulases used in the disintegration of cell wall of fruits, releasing the juice and making it easier to extract (Shariq & Sohail, 2019). This process improves the yield and quality of the juice, resulting in a product with a better taste and texture. Another important application of microbial cellulases in the food processing sector in the production of dairy products. These enzymes are used to break down the cellulose in the plant material used to feed cows, resulting in a better-quality milk. Cellulases can also be used to enhance functional characteristics of cheese, making it more palatable for consumers (Ejaz et al., 2021).

Transglutaminases

These enzymes crosslink proteins to form a stronger bond. Transglutaminases are used in the meat industry to create meat products with improved texture and juiciness. They are also used in the production of cheese, where they improve the texture and melting properties of the cheese (Akbari et al., 2021). The enzyme transglutaminase can be applied to fruits and vegetables as a coating to help maintain their freshness. In a particular study, applying transglutaminase to cut celery not only preserved its freshness, but also decreased bacterial growth (Manessis et al., 2020). Transglutaminases have applications in dairy products, such as cheese and yogurt. They can help to increase the firmness of cheese and improve its sliceability. In yogurt, they can help to improve the viscosity and stability of the product (de Góes-Favoni & Bueno, 2014). Transglutaminases are used in the manufacturing of bakery products, such as rusk and cakes. They can help to enhance the texture and volume of the food items. Furthermore, they are used to improve the texture and functionality of seafood food items, such as fish fillets and surimi. They can help to increase the firmness and gelation properties of these products. Transglutaminases are also used in the production of plant-based products, such as meat substitutes and dairy alternatives. They can help to improve the texture and functionality of these products, making them more similar to their animal-based counterparts.

Lactase

Lactases are a type of enzyme that has the ability to decompose lactose, which is the natural sugar present in dairy products and milk, into simpler forms of sugar such as glucose and galactose. In food processing industry, lactases are mainly utilized for the production of dairy products that are either lactose-free or have lower lactose content. This has gained popularity because many people are lactose intolerant (Sutay Kocabaş et al., 2022). Lactase is used in the dairy industry to improve the quality of dairy products. It can help to reduce the crystallization of lactose, improve the texture, and enhance the flavor of dairy products. Lactase is also used in the

production of infant formula to make it easier to digest for babies who are lactose intolerant or have difficulty digesting lactose (Oak & Jha, 2019). Lactase is used in the baking industry to improve the texture and flavor of baked goods, such as bread, cakes, and cookies, synthesis of lactose. Lactase is also used in sports nutrition products to improve the digestion and absorption of lactose in athletes who consume large amounts of dairy products as part of their training diet (Odell & Wallis, 2021). Overall, the application of lactase in the food processing has several applications, in the production of lactose-free products, improving the quality of dairy products, and enhancing the digestibility of lactose in various food products.

Application of Enzymes as Food Antioxidants

Enzymes are not only essential for biological functions like digestion and metabolism, but they also have effective role in food preservation and antioxidant applications. Acting as natural antioxidants, enzymes can prevent food spoilage and enhance the shelf life of products by breaking down harmful substances. Certain enzymes, like catalase and superoxide dismutase, can specifically break down hydrogen peroxide and superoxide radicals, respectively, to prevent oxidative damage to food (Zeb, 2020). In food preservation, enzymes like lactase and peroxidase can be used in meat products to prevent lipid oxidation, which leads to spoilage (Bensid et al., 2022). Additionally, enzymes such as ascorbate oxidase and polyphenol oxidase can be utilized in fruits and vegetables to prevent browning and increase shelf life. Enzymes also have a place in food processing, where they can improve texture and flavor, such as tenderizing meat with papain and bromelain, or improving cheese and yogurt quality (Carocho et al., 2014). Overall, enzymes have a broad range of uses in food preservation, and quality improvement due to their natural antioxidant properties. With further research, the food industry can effectively use enzymes to produce healthier, higher-quality food products.

Enzymes Applications Dairy Industry

Enzymes have a vital role in the dairy industry, serving various stages of production. Cheese making heavily relies on enzymes, with rennet being a commonly used mixture that curdles milk to form curds (Mir Khan & Selamoglu, 2020). Proteases are also used during the aging process of cheese to break down proteins and fats, creating different flavors and textures for each type of cheese. Milk processing also benefits from enzymes, enhancing the quality and taste of dairy products. For example, lactase is a widely used enzyme that synthesis lactose in milk, making it tolerable for lactose intolerant individuals (Facioni et al., 2020). Proteases are also employed to enhance the flavor of certain dairy products. Enzymes are also essential in yogurt production, where lactase is the primary enzyme used to convert lactose in

milk into glucose and galactose, creating a smoother and thicker texture. Other enzymes are also used to improve the texture and taste of yogurt (Sutay Kocabaş et al., 2022). Whey, a by-product of cheese making, can also be utilized through the use of enzymes. Enzymes can break down lactose and protein in whey to produce whey protein concentrate, which is high in protein and used in various food products, including nutrition bars and sports drinks. Overall, enzymes are essential in the dairy industry, with applications in cheese making, milk processing, yogurt production, and whey processing. These enzymes can help create high-quality dairy products with unique flavors and textures while also improving their nutritional value, ultimately satisfying the demands of consumers (Rocha & Guerra, 2020).

Enzymes Application in Meat Industries

Enzymes play a crucial role in the meat industry as they can significantly improve the quality, taste, and shelf life of meat products. In particular, enzymes like papain, bromelain, and ficin are used to tenderize meat by breaking down collagen and connective tissues present in muscle fibers. By doing so, the texture and tenderness of the meat are enhanced, resulting in a more enjoyable eating experience (Singh et al., 2018). Moreover, proteases, another type of enzyme, are used to enhance the flavor of meat. These enzymes break down proteins into smaller peptides and amino acids, contributing to the savory taste and aroma of cooked meat. Preservation is also an essential application of enzymes in the meat industry. Lactoperoxidase and glucose oxidase are examples of enzymes used for this purpose as they stop further growth of bacteria and maintain the color, organoleptic properties of meat products (Raveendran et al., 2018). In meat processing, enzymes like transglutaminase are utilized to bind meat pieces together, resulting in a uniform product such as sausages, meatballs, and burgers. Transglutaminase is particularly useful in creating meat products that have a consistent texture and shape, making it easier for manufacturers to produce a high volume of identical products (Kieliszek & Misiewicz, 2014). Lastly, enzymes like lipases are used to decrease the fat content in meat products by breaking down fat molecules into smaller components. This is particularly useful for consumers who are health-conscious and want to reduce their fat intake. By using enzymes, manufacturers can create leaner meat products without compromising their flavor or texture (Chandra et al., 2020).

Enzymes have various applications in the meat industry, and their use has become increasingly important for manufacturers who want to improve the quality, taste, and longevity of their meat products. The various enzymes used in the industry serve specific purposes, such as tenderization, flavor enhancement, preservation, meat processing, and fat reduction. By using enzymes, the meat industry can continue to innovate and provide consumers with high-quality products that meet their changing needs and preferences.

Application of Immobilized Enzymes in Food Industry

Immobilized enzymes have various applications in the food industry. They are used for producing high fructose corn syrup, wine, cheese, bread, beer, and fruit juices. These enzymes help in breaking down complex carbohydrates, coagulating milk, and breaking down pectin (Homaei, 2015). The use of immobilized enzymes improves the efficiency and quality of food production while reducing the cost and environmental impact of the processes (Yushkova et al., 2019). Immobilized enzymes have become increasingly popular in the food industry due to their advantages over free enzymes. Immobilization is a process in which enzymes are bound or trapped onto a solid support, such as a matrix or a carrier material, to create a stable and reusable enzyme system. One of the main benefits of immobilized enzymes is their increased stability, which allows for better performance and longer lifespan. They also offer improved control over enzyme activity, which is critical for precise reaction conditions and product quality. Additionally, making product recovery and purification more efficient. In the food industry, immobilized enzymes have broad range of use, such as improving food quality, enhancing process output, and reducing costs. For instance, immobilized enzymes can be used for the manufacturing of high-fructose corn syrup, which is a sweetener commonly used in the food industry. These Enzymes can also be used for the production of wine, beer, and cheese, as well as for the hydrolysis of proteins to produce bioactive peptides. Immobilized enzymes are also used in the production of fruit juices, where they can improve the yield and quality of the juice, as well as reduce processing time and costs. In the baking industry, immobilized enzymes are used to modify flour characteristics, resulting in improved dough handling properties and better texture of baked goods (Swaisgood, 2002).

Application Enzymes in Food Industry

The food industry encompasses a broad range of dairy products that includes various items such as milk (Fischer et al., 2011). Coagulants derived from acid proteases specifically chymosin (EC 3.4.23.4), are utilized in cheese production along with other enzymes. Chymosin is responsible for approximately 20–30% of milk coagulants that are utilized globally. Cheese maturation is facilitated by proteinases which expedite the process of protein breakdown, a crucial biochemical event that greatly influences the texture and flavor of cheese. Moreover, peptidases are employed to eliminate the bitter taste resulting from protein breakdown during cheese maturation (Hati et al., 2013). The types of cheese estimated by the process of maturation, which results in unique textures and aromas. During the maturation process, Lipase synthesis the triglycerides to simple glycerol lead to development of flavour in cheese. The use of lipases in the maturation process can accelerate the process by two to five times, but it is crucial to carefully regulate the amount and

activity of the enzyme, as excessive lipases can cause rancidity and a decrease in cheese yield (Chandra et al., 2020). The meat industry faces a significant challenge in improving the sensory qualities of meat products. Proteolytic enzymes can be used to tenderize meat without the use of chemicals and brines, and while papain and ficin have been used, transglutaminase is the most widely utilized enzyme. This enzyme, first produced by *Streptoverticillium mobarence* in the early 1980s, offers advantages over other enzymes due to its low cost and ability to enhance nutritional value by adding essential amino acids to protein matrices. Transglutaminase also improves the texture, firmness, elasticity, and emulsification of meat products like sausages, resulting in higher quality and greater variety. In combination with proteases, thermolysin is employed to produce food protein and hasten the ripening of dry sausages (Cobos & Díaz, 2015). When it comes to making fermented beverages like wine and beer, the main priorities are optimizing the process, improving yield, and maintaining or enhancing colors and flavors. Enzymes can help achieve these goals while also reducing the calorie and sulfur content of beer and improving wine clarity. For example, sulfhydryl proteases can enhance clarity and remove butter odor in beer caused by diacetyl. β-glucanases are used to reduce beer viscosity through β-glucan hydrolysis. Pectinases play a critical role in wine and beer production by facilitating extraction and filtration, improving juice yield, flavor, odor, and clarification. Although fruits and vegetables contain pectinases with low activity, the industry typically uses microbial enzymes because of their stability and resistance to fermentation conditions. For flavored beverages, juice producers must address issues related to stability, quality, clarification, viscosity, and yield during production to ensure consumer acceptance. The nutritional value of food products has become increasingly important to consumers. Enzymes such as α-galactosidase and phytases are commonly used to improve food products quality, particularly those made from legumes and cereals. Phytate is known to reduce the bioavailability of nutrients by binding with positively charged proteins and chelating divalent and multi-valent cations. Phytase efficiently dephosphorylates phytate, releasing minerals and increasing their bioavailability. The development of hypoallergenic foods is another potential market area. Antigenic proteins found in wheat, peanuts, soybeans, chickpeas, milk, and eggs can cause allergic reactions. Enzymes such as proteases, actinase, alcalase, flavourzyme, and neutrase can help reduce allergens, providing a health benefit. Enzymes are also used in the food industry to monitor process quality parameters. Biosensors that use enzymes can tightly combine biorecognition elements and physical transducers to monitor and detect target compounds (Vashishth et al., 2017).

Role of Microbial Enzymes in Food Analysis

Microbial enzymes play a critical role in food analysis as they can be used for the detection and quantification of various food parts like carbohydrates, proteins, lipids, and enzymes themselves. They can also be used to assess food quality,

freshness, and safety. Protease enzymes produced by microorganisms such as bacteria, fungi, and yeast can be used to analyse protein content in food. For example, the enzyme trypsin can be used to hydrolyze proteins in food samples, and the resulting peptides can be analysed using advance techniques to determine protein content. Protease enzymes have a important role in food analysis as they used to determine the protein content of food and to identify specific proteins of interest. Protease enzymes have a range of applications, including food processing, quality control, and safety assurance. In food analysis, protease enzymes disintegrate protein to peptides, which can then be quantified using various analytical techniques such as chromatography or spectroscopy. Protease enzymes such as trypsin, pepsin, and papain are commonly used for this purpose (Panchaud et al., 2012). One application of protease enzymes in food analysis is the determination of protein content in food samples. The most commonly used method for determining protein content in food is the Kjeldahl method, which involves the digestion of proteins with sulfuric acid and the subsequent quantification of the resulting ammonia. However, this method can be time-consuming and requires specialized equipment. Alternatively, protease enzymes such as trypsin can be used to digest proteins in food samples, and the resulting peptides can be quantified using analytical techniques such as HPLC or MS (Arte et al., 2015). Another application of protease enzymes in food analysis is the identification of specific proteins in food samples. Protease enzymes can be used to digest proteins into smaller peptides, which can then be analysed using mass spectrometry or other techniques to identify specific proteins. This approach can be useful for detecting allergens, contaminants, or other specific proteins of interest in food samples. Overall, protease enzymes play a crucial role in food analysis, enabling the determination of protein content and identification of specific proteins of interest in food samples (Schlüter et al., 2008).

Amylase enzymes produced by microorganisms can be used to analyze carbohydrate content in food. For example, the enzyme alpha-amylase can be used to hydrolyze starch in food samples, and the resulting glucose can be quantified using techniques such as colorimetry or HPLC (Hall, 2015). Lipase enzymes produced by microorganisms can be used to analyze lipid content in food. For example, the enzyme lipase can be used to hydrolyze triglycerides in food samples, and the resulting fatty acids can be quantified using techniques such as gas chromatography (GC) or HPLC. ELISA is a technique that uses antibodies to detect and quantify specific food components. Microbial enzymes can be used to produce these antibodies, which can then be used in ELISA assays to detect food allergens, pathogens, or contaminants (Chandra et al., 2020).

Conclusion

The current study, the use of microbial enzymes has brought about significant advancements in the food processing sector by offering several benefits in food production and preservation. These enzymes are essential in the production of

various food products such as dairy, meat, bread, and beer, among others. They have proven to be superior to traditional enzymes because of their specificity, cost-effectiveness, and ability to ensure precise control over the final product's quality. Additionally, they are more environmentally friendly and sustainable compared to chemical catalysts, which pose health and environmental risks. The increasing demand for safer, healthier, and more sustainable food products will continue to drive the adoption enzymes derived from microrgansims.

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Chapter 14
Impact of Water Contamination on Food Safety and Related Health Risks

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Introduction

The recently published first report on the State of Global Water Resources by the World Meteorological Organization states that the change in climate, and environment has an impact on global water resources (WMO, 2022). The focus should be on monitoring and management of global freshwater resources where the demand is fast growing but the supplies are limited. WMO Secretary-General, Prof. Petteri Taalas stated that water can be a parameter to study the impact of climate change. More intense and frequent droughts, extreme flooding, erratic seasonal rainfall, and accelerated melting of glaciers are seen across the globe. Water is a fast-depleting resource and its availability is critical to the human race. It impacts not only human health but the entire food chain. The chapter includes the significance of water as a resource, its use in food production and processing, highlighting different levels. It also covers the source of contamination of water and associated diseases with the detection methods. Treatment of water and strategies for improving the water quality in the food sector are also discussed in detail.

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Water as a Resource

The availability of water which is clean and safe rates highest in the current Environment, Social, and Governance (ESG) concerns. Freshwater is a fast depleting resource in spite of it being renewable. The reasons are increasing human population, urbanisation, industrialization leading to high water demand and consumption. The majority of land sources of water are getting polluted. The United Nations report states that a 40% shortfall in water supply will occur by 2030 and it will increase to five billion by 2050 if this crisis will not be handled then (Alexandratos & Bruinsma, 2012). According to the UN, the climate and development goals are achievable only if water is at the centre of adaptation strategies.

Boretti and Rosa (2019) have stated that, in the next 20 years, the need for water will multiply in all sectors of society, like agriculture, commercial, and domestic. The rate of the commercial and domestic sectors will be more than the agricultural, however, the agricultural sector will still be the biggest consumer of water resources. Currently, worldwide agriculture accounts for 70% of the total water, which is majorly used for irrigation. It is estimated that by 2050, the food demand will escalate by 60% leading to a need for more arable land and increase in production which is only possible if there is enough water to meet these requirements.

Role of Water in the Food Sector

Food systems and water are interdependent. Water plays an essential role in the activities pertaining to food production and as well as processing (FAO, 2017) right from the primary production at the farm level (growing crops, rearing animals, and in aquaculture), food processing, and food consumption. Water is a crucial resource as its use spans all across the food sector with a long list of applications. As water plays a crucial part in hygiene and sanitation, hence it comes in contact with food directly or indirectly.

Water resources are quickly depleting globally and not all the stakeholders have access to clean and safe water. The use of water needs a conservative approach and approaches must focus on the reuse of water if it does not pose a health concern for consumers.

Each country, region, area, and food industry have a different requirement in terms of its water resources and their quality. A good quality water is a prerequisite at all levels. But if there is a variation in the quality of water, it can still be used for different purposes.

Apart from quality of water, an in-depth understanding of the role of water in food safety is needed, as to avoid illness or outbreaks. The physical, chemical and microbiological parameters of water affect food safety. Common contaminants of water such as pathogenic bacteria, and viruses, may enter the food chain and affect food safety. Even the presence of physical hazards such as glass and metal particles in water can pose a threat to the safety of food and these may cause serious harm to consumers.

The usage of water in food production can be classified into four categories in a broad sense: (1) primary food production, (2) cleaning and sanitation, (3) processing operations, and (4) as a food ingredient.

Water in Primary Food Production

Human population across the globe depends mainly on Agriculture, including animal husbandry and forestry, for their food. Livestock and aquaculture provide an array of food products such as meat, poultry, dairy products, and fish, or seafood to the human race. But growing plants as well as rearing livestock requires a large amount of water. Thus, water demand in agriculture is 100 times more than domestic water demand. In fact, this makes agriculture to be the largest consumer of water for growing the food to meet the needs of millions.

Largest proportion of water is used for crop irrigation purposes followed by watering of livestock, cleaning of equipment and maintenance of general hygiene of the animals. Studies have shown that the production of meat requires at least 6–20 times more water than the production of crops. The crop patterns need large quantities of water.

Crop production requires large quantities of water but at the right time of the growth cycle to ensure high yield as different agricultural crops have very specific water needs depending on the climatic conditions. The studies by Oosterhuis and Bachus in 2014 have shown that the cereal grains, oilseeds, legumes, roots, and tubers utilise less water for growth in comparison to livestock production.

Water in Food Processing

Use of water is an integral step in the food processing industry. Majority of the operations in the food sector utilise water be it be washing, grading and sorting in the beginning of the process to formulation of the food product, reconstitution, pasteurisation, generation of steam for heating purposes, cooling, cleaning (both CIP as well as COP) and sanitization purposes. Every industry also requires mandatory water resources in the premises as part of the fire safety guidelines.

In current times, the recycling of water for reuse is a required step towards sustainability due to increasing scarcity of this resource on our planet. This requires greater focus in the case of the food industry as the water requirements are very high and unavoidable. Majority of food processing operations require water in one or other form so recycling is essential. Recycling helps in water conservation, reduction in cost, and provides assurance of a secure and continuous water supply. Keeping water conservation in view, the governments in various countries have permitted the use of recycled water in the food processing or an ingredient of food if it meets the same standards of drinking water. There are few instances where the clean

seawater is permitted for use by the authorities in the seafood sector although it is non-potable in nature. The use of sea water is limited to basic processing steps such rinsing and washing of seafood which usually includes whole fishes and shellfish.

Water in Cleaning and Sanitation

Cleaning and sanitation in a food sector are essential programs for production of safe and hygienic food. Cleaning is a prerequisite for increasing the effectiveness of sanitation process as dirty surfaces cannot be sanitised properly. In the food sector, apart from the washing of raw food material, cleaning includes washing of utensils, appliances, equipment, gadgets, vessels, associated machinery, contact surfaces and even the cleanliness of food handlers, which uses water. Cleaning means removal of dirt, soil and other organic material like food debris/ waste, grease, fat, and protein contamination etc. from all the surfaces that come in contact with/ met/ encounter food directly or indirectly and can promote microbial contamination and growth. This can be done manually by scrubbing or by adopting other ways of cleaning including clean-in-place (CIP) systems, clean-out-of-place (COP), use of high pressure, spraying, foaming etc. The quality of water used for cleaning and washing is of great concern. It should be potable and as per ILSI 2008 the total plate count should be lesser than 500 per ml while coliform should be less than 1 and psychotropic less than 10 per ml.

Sanitation means reducing the number of pathogens or spoilage organisms on a cleaned surface to a safe level by applying physical or chemical agents. As per Association of Official Analytical Chemists five log reduction (99.999%) can be achieved in contamination level of contact surfaces in 30 seconds. Seven step approach can be used for cleaning and sanitation which involve physical removal of soil, rinsing with warm good quality water, use detergents and scrubbers to remove/ take off fat and proteinaceous debris, final/thorough rinsing with microbiologically safe water to remove all residues, inspect cleaned surfaces for any residue, application of sanitizers/ disinfectants and finally drying. Most of these processes require water which remove the dirt and debris from the contact surfaces and act as a medium for detergent and sanitisers. As use of contaminated water decreases the efficacy of disinfectants and sanitisers, properly treated pathogen free water should be used for cleaning and sanitation.

Water as a Food Ingredient

Water is an integral component of food. More than 70% of many fresh foods like meat, vegetables, fruits etc. is constituted by water that can be present in free, adsorbed or bound form. It is an essential ingredient which is used in routine for food preparation, cooking, and consumption. Water is also used as a vehicle during

preservation and storage of food and is a major constituent/ element in many consumed forms of food like fruit juices, soups etc.. Water is a commonly used diluent and universal solvent to dissolve and extract compounds from various food items. It determines/controls the palatability, texture and appearance of food, results in gelatinisation of starch and also supports chemical and enzymatic reactions in food. Besides, water is used in production of food, making ice, for drinking, cleaning and sanitising food environment and equipment as well.

The quality of water used in the food sector is very important as it influences the food quality and safety. Water can be obtained from different sources and may be distributed by government agencies or private distributors. Very high quality and adequate supply of drinking water is mandatory to produce healthy and safe food. Low quality of water with a bad colour, odour, taste, and impurities can result in injury or illness and low-quality products. Often the water quality is ignored in food production systems, although maintaining it is important, as it can affect the products and operations. Mismanagement of water and its poor quality can result in bad quality and unhygienic food, economic loss, and poor management and operations of various equipment in the food industry. There is a need that at every step of food production strict criteria to be followed for water analysis.

Sources of Water Contamination

Contamination of water not only concerns humans but marine and wildlife too. There are various sources of water pollution such as physical, chemical, and biological. Contaminants are derived from various point and nonpoint sources. Point sources are specific to a location such as mines, power plants, factories, municipal discharges, etc. A nonpoint source is a contamination caused by a wide area or various diffused sources like watersheds or waste from land to waterways (Schweitzer & Noblet, 2018; Calderon, 2000; Denchak, 2022).

Majorly, the problem of water pollution is faced by low and middle-income countries (Bain et al., 2014), although water contamination caused by one country can spread to others, known as transboundary. It can cause a worldwide disaster in cases like oil spills, etc. (Denchak, 2022).

Physical Contaminants

Water streams that are not treated by methods like sedimentation and filtration consist of physical contaminants. These contaminants affect the physical properties of water like appearance, odour, etc. (US EPA, 2014). Major contaminants came from sewage, river dumping, and marine dumping, but microphysical particles (size 1–1000 μm) like microplastics, glass, and metal microparticles can cause major physiological dysfunctions in humans and animals when consumed.

Sewage Wastewater from households and industries carry various contaminants, chemical, biological and physical. Domestic sewage contains toilet paper, soaps, detergents, food waste, sanitary napkins, tampons, etc. Whereas industries' releases can be highly toxic and may contain toxic sludge, adhesives, paints, and by-products. Sewage also includes stormwater runoff consisting of road oil, grease, and debris. Wastewater also includes microplastics like polyethylene and polypropylene beads (Gatidou et al., 2019) from clothes, bedding fabrics or plastic packaging. According to the United Nations, almost 80% of the world's sewage remains untreated and flows back into the environment (Denchak, 2022).

Agriculture Runoff The agriculture sector consumes the largest amount of freshwater for activities like farming and livestock, but it also causes water pollution. Slurries, animal faecal waste, and manure are the main constituents of water contaminants from agriculture. Spills caused by milk dairies also disturb the water quality (Denchak, 2022).

Oil Spill It is a disastrous condition environmentally and economically, which highly affects the water quality. The liquid petroleum is released in the environment, mainly in marine environments. As the oil is not soluble in water hence it forms a layer on the water, the oxygen supply is hindered for the marine ecosystem and causes deaths of the organisms (Doty, 2022; Wikipedia contributors, 2022). Oil spills occur during the transportation, or purposeful dumping of petroleum products. Oil spills can also occur on land due to a lack of storage facilities or from dripping from automobiles.

Marine/River Dumping In spite of this practice being banned, large amounts of waste including plastics, garden cuttings, electronic waste, etc. are deliberately dumped in coastal waters. This marine debris decreases the oxygen levels in the water. Some of these materials take more than 100 years to degrade and can cause flooding and harm to wildlife (Doty, 2022).

Chemical Contaminants

For the growing need for energy production, technologies like horizontal drilling and hydraulic fracturing have helped the human race but, on the other hand, have caused a hike in water contamination. Industries, mines, and nuclear power plants release radioactive waste and groundwater can be polluted with heavy metals (e.g., nickel, mercury, copper, and chromium) which can have serious carcinogenic effects (Wongsasuluk et al., 2014). By-products of pharmaceuticals are toxic and cause water pollution if not treated before discarding (Shen & Andrews, 2011). During the rain, agricultural runoff containing fertilizers, pesticides, antibiotics, hormones, salts, and heavy metals from livestock excretion, can contaminate the canals and rivers. Chemical contaminants in water for irrigation can affect food production and

can cause severe health problems based on the type of contaminant (Malakar et al., 2019). The list of chemical contaminants is mentioned below in Table 14.1.

Biological Contaminants

The presence of disease causing organisms and their toxins in the water bodies causes water pollution and can result in severe illness. These microorganisms can be bacteria, protozoans, viruses, or fungi and toxins like cyanotoxin and mycotoxin, that are released by cyanobacteria and fungi respectively. Mostly these microbes enter the water stream by sewage or agriculture runoff that includes animal and human faecal waste, rather than growing in water. Although there are some microbes that have the ability to grow in water when the conditions are suitable, like water temperature, oxygen level, nutrient level, etc. (Bhagwat, 2019). Increased levels of nutrients can cause algal blooms known as eutrophication, which in turn decrease the oxygen level and can also form neurotoxins in water which affect marine life.

The most common pathogens present in water are; bacteria like *Legionella, Escherichia coli, Campylobacter, Salmonella, and Shigella,* which causes diseases like cholera, salmonellosis, shigellosis, leptospirosis; viruses like Enterovirus, Norovirus, Norwalk virus, Rotavirus, Human enteric viruses, Hepatitis A virus, which causes liver inflammation, dengue fever, vomiting, diarrhoea; protozoa like *Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum, Plasmodium,* which causes diarrhoea, malaria, stomach cramps, nausea; helminths like Schistosomes, Fasciola hepatica, which causes schistosomiasis, fascioliasis (Bhagwat, 2019).

Health Hazards in Water

Water is the basic requirement for life sustainability that gets polluted by various contaminants such as heavy metals, animal and human faecal matter, untreated sewage, and chemical effluents from industries like heavy metals, nitrates, fluorides, iron, salinity, and arsenic, etc., and cause health hazards (Table 14.2). About 80% of industrial waste enters into water bodies without any pre-treatment. Moreover, water is an integral resource of various food-related activities starting from food production, through preparation and processing to its consumption. Water and food quality are interrelated. Unsustainable agriculture practices like overuse and leaching of fertilisers and pesticides, agricultural runoff, and domestic wastewater can enter into various water sources viz. groundwater, rivers, ponds, lakes, swamps, etc., and deteriorate water quality. In water-stressed areas, wastewater is used in various food system activities, cleaning of raw materials, equipment and food contact surfaces, washing of utensils for drinking, etc. The use of unclean water and exposure to unclean environments introduced pathogenic microorganisms in food and may have ill effects on human health (Bhagwat, 2019; Linderhof et al., 2021).

Table 14.1 Chemical contaminants in drinking water

Chemical contaminants	Point of entry	Effects caused	References
Bicarbonates, chlorides or sulphates (sodium, calcium or magnesium)	Carbonates from sedimentary rocks (limestone and chalk), seepage, and runoff from soils. Chloride can be from salt-bearing geological formations, salt used for road de-icing, from wastewater. Sulphates from volcanic eruptions, mineral weathering, and sea spray aerosols. Acid mine drainage, fertiliser leaching from agricultural soils.	Increases water hardness; scale formation; corrosion	Netsol Water Solutions Pvt Ltd (2021), Zak et al. (2021), Bhagwat (2019)
Iron, copper, manganese	Rocks and minerals, or iron and steel pipes. Discharge of acid industrial wastes or mine drainage	Filming; staining; corrosion	APS Water Services Corp (2023), Bhagwat (2019)
Heavy metals (arsenic, radon, uranium, lead)	Industrial and mine wastewater discharges	Biomagnification; high morbidity and mortality rates	Linderhof et al. (2021)
Pesticides and fertilisers (nitrogen and phosphorus)	Agricultural runoff; sewage (detergents and soaps)	Eutrophication; increase toxicity	US EPA (2013)

(continued)

Table 14.1 (continued)

Chemical contaminants	Point of entry	Effects caused	References
Perfluoroalkyl and polyfluoroalkyl substances (PFAS)	Septic systems; cosmetics; paints and varnishes; cleaning products; firefighting foam	Adverse reproductive, developmental, and immunological effects in animals and humans	Schaider et al. (2016), "Perfluoroalkyl and polyfluoroalkyl substances (PFAS) in drinking water" (2016), "Potential health effects of PFAS chemicals" (2022)
Organic chemical pollutants (dichlorodiphenyltrichloroethane – DDT; polychlorinated biphenyls – PCBs, etc.,)	Used as pesticides and insecticides hence are sprayed into the air and on soil or water (DDT was also sprayed in the environment to control mosquitos); PCBs are released in the environment through spills, leaks from electrical and other equipment, and improper disposal and storage; plastic and utensils making industries	Aquatic system depletion; eggshell thinning and embryo deaths in birds; health issues in animals and humans	American Chemical Society (2019), "Polychlorinated Biphenyls (PCBs)" (2009), Nanseu-Njiki et al. (2010)

The contamination of water and also of food is a big safety concern and pose serious health risks. Various bacteria like *Shigella, E. coli, Salmonella, Vibrio, Campylobacter jejuni*, different viruses like Hepatitis A, polio virus, and protozoans like *Giardia lamblia* may present in water and become the source of infection. These pathogens and chemically contaminated water on consumption result in various waterborne diseases and are responsible for high mortality and morbidity in affected individuals. Hundreds of millions of people die every year because of water-related diseases particularly in developing countries where accessibility to safe water may be poor. Waterborne diseases are also the cause of several food-related outbreaks and infections that are inevitable when drinking/cooking with

Table 14.2 Common chemical contaminants and their health effects

Chemical	Health effects
Fluorides (F^-)	Causes nausea, abdominal cramps, haemorrhagic gastroenteritis, and paralysis of the respiratory system leading to death. Chronically affect teeth and bones, anaemia, weight loss (fluorosis)
Lead (Pb)	Interfere with enzyme functions, severely affect kidney, nervous and reproductive system, cause anaemia by reducing haeme synthesis, elevated blood pressure, miscarriage during pregnancy, (lead poisoning)
Arsenic (AS)	Result in vomiting, stomach ache, diarrhoea, muscle cramps and death. On longer exposure cause cancer of organs such as the bladder, lungs, kidney, and skin (Arsenicosis)
Chemical pollutants and disinfection by-products (DBPs) ex. chlorinated solvents, methyl tert-butyl ether (MTBE)	Break DNA, damage liver, & kidney damage cause neurological problems and cancer of bladder, colon, brain etc
Pesticides ex. DDT, organophosphates	Skin irritation, impairment of endocrine and nervous system functions, cause cancer
Perchlorate (ClO−4)	Disturb iodine uptake and thyroid functioning, may have toxic effect on respiratory system and cause reduction in blood cell synthesis by bone marrow
Nitrates (NO_3^-)	Transform to nitrites by gut microbes that can that results in methemoglobinemia, short pregnancy, headaches, respiratory problems, and, ultimately death
Radioactive wastes	Internal tissue damage, haemorrhage, cardiovascular and nervous tissue damage, cancer, radiation sickness

such unclean water. These infectious ailments may result in malnutrition, reduced immunity, a high risk of recurrent diarrhoea, and mortality, especially in infants, young children, the elderly, and people with comorbidities like diabetes, and heart and kidney diseases. About 90% of diarrheal diseases and about 25% of hospital admission globally, particularly in developing countries, are waterborne. The risks are more in rural populations. These waterborne diseases are responsible for about 5 lakh deaths of people each year, as estimated by the World Health Organisation (WHO). Diarrhoea is reported to be chief cause of death in children of age group below 5 years.

Waterborne Diseases and Control Measures

Unavailability of safe clean water for drinking and cooking and unhygienic/unsanitary conditions, particularly many in underdeveloped countries, may lead to the breakout of waterborne diseases. The prominent symptoms are diarrhoea, abdominal pains, dehydration, fever, nausea, vomiting, headache, muscle ache, and loss of

appetite. A clean environment along with good hygienic and sanitary conditions prevent their spread. Person-to-person transmission is generally very rare. Some of the common waterborne diseases (Adams & Moss, 2000; Frazier et al., 2013) occur on ingestion of water and food contaminated with disease-causing microorganisms (bacteria, viruses, etc.) include:

Traveller's Diarrhoea and Haemorrhagic Colitis Traveller's diarrhoea is the most common disease caused by *Escherichia coli*. Though most of the strains of *E. coli* are harmless, certain strains present in water sources result in diarrhoea, dysentery, vomiting, stomach pain, and sometimes serious infections like haemorrhagic colitis. In most cases, symptoms are mild and resolve within a week but life-threatening symptoms may develop in new-borns, young children, older people, and people with low immunity. The haemolytic uremic syndrome may occur on infection with the O157:H7 strain which can lead to kidney failure and death. The diseases of *E.coli* can be prevented by avoiding the use of water contaminated with animal and human faeces, proper washing of fruits and vegetables, thorough cooking of meat and food, and washing hands. Taking rest, plenty of water and medication help in treating the disease.

Giardiasis Giardiasis and cryptosporidiosis are the second most common gastrointestinal tract illnesses associated with water. These spread through water or food contaminated with parasites or their cysts. Public water bodies, lakes, swimming pools are the common sources for infection transmission and disease is quite common in cramped places with poor sanitation. The cause of giardiasis is *Giardia lamblia,* common waterborne pathogen presents worldwide, and convicted in many municipal waters associated outbreaks. The organism is not invasive. The symptoms are diarrhoea, abdominal discomfort, pain, bloating, flatulence, nausea, vomiting, loss of appetite and weight, extreme tiredness, fatigue, etc. Diagnosis involves looking for Giardia's presence in stool samples or through enteroscopy. Anti-parasitic drugs can be administered for treatment. The disease/illness appears after 1–4 weeks of exposure and persist for several weeks.

Cryptosporidiosis The disease is caused by the parasite *Cryptosporidium* on ingestion of stool-contaminated uncooked food, swallowing contaminated water during bathing or swimming, or contact with contaminated surfaces. The disease is everywhere but more frequent in rural areas with poor sanitation. More common symptoms are fever, vomiting, stomach cramps, loose or watery stools, and weight loss with slight variations with each individual. Dehydration is a common complication. The symptoms persist for about 2 weeks but parasites remain in stool for about 2 months, thus the patient can spread even after the resolution of symptoms. Generally, the disease is not severe but life-threatening consequences can occur in individuals with weak immunity (cancer, HIV, transplant, etc.). Diagnosis is done by seeing parasites in stool samples. Medication may be needed in cases of severe infections.

Amoebiasis Amoebic dysentery, salmonellosis, and bacillary dysentery are the third most common waterborne diseases after travellers' diarrhoea, giardiasis, and cryptosporidiosis. Amoebic dysentery caused by cysts of a protozoan, *Entamoeba histolytica*, can occur on the consumption of faecal-contaminated food and water. The patients suffer from intestinal inflammation, severe abdominal cramps, and dysentery (blood and mucus in stool) due to ulceration of the colon. Other symptoms include fever, chill, nausea, vomiting, fatigue, and weight loss. Dysentery lasts for about 3–7 days leading to dehydration. As many as 50 million cysts may be passed by the infected person. Diagnosis can be done through blood and stool tests. The presence of cysts indicates infection. Liver function tests to know liver damage as well as a colonoscopy may be recommended in severe cases. The treatment typically involves replenishment of fluid loss through rehydration, OTC medication, and the use of antibiotics if need be.

Shigellosis or Bacillary Dysentery Another bacterial disease transmitted through the faecal oral route is shigellosis caused by various *Shigella sp.* viz. *S. sonnei, S. flexneri, S, dysenteriae*, and *S. boydii* present in contaminated food and water. Pathogenicity is because of lipopolysaccharide endotoxin that affects the mucosa of the intestine. On reaching the intestine, the bacterium causes high fever, frequent diarrhoea with blood in stool, severe abdominal cramps, nausea & vomiting and excessive thirst within 1–7 days of ingestion. Rehydration is required to replenish the fluid loss. Medication and antibiotics can be recommended depending on the severity.

Salmonellosis and Typhoid These are bacterial infections caused by *Salmonella bongori*, *Salmonella enteric* and *S. typhi* respectively that are spread through faecal contaminated food and water. The incidences increase under conditions of low personal hygiene and unsanitary conditions. The disease symptoms appear within 12–36 hours in salmonellosis and after 7–28 days in typhoid. Common symptoms are high fever, muscular pain, headache, excessive weakness, loss of appetite, stomach pain, constipation, cough, skin rashes, weight loss, chills, and diarrhoea. In typhoid, the bacterium enters through the faecal-oral route and passes to the bloodstream through the intestinal wall, and then enters various organs. The disease can be diagnosed by the presence of *Salmonella* in blood, stool, or urine samples or by looking for the antibody presence through the WIDAL test. The disease can be prevented through regular hand washing and having food in clean places. Injectable and oral vaccines are available for children and people traveling to high-risk areas. Treatment involves timely/early medical care, administration of antibiotics, and keeping the patient rehydrated through oral fluids. Surgical treatment may be required in more advanced stages.

Cholera Ingestion of *Vibrio cholerae* causes the disease cholera which is frequently transmitted by consuming roadside food prepared unhygienically or raw vegetables and raw fish grown in dirty water. As per WHO, there are approximately four million cases of cholera reported each year. On ingestion, bacteria move to the intestine where it produces toxins resulting in symptoms of cholera. The disease is

characterized by abdominal pain, loose frequent stool (severe diarrhoea), nausea, vomiting, feeling thirsty, dehydration, and muscular cramps. In some cases, the disease can be life-threatening. Diagnosis is through clinical symptoms including physical examination for other signs like reduced elasticity of the skin, the dry membrane of the nose, eyelids, mouth and travel history to places where cholera can spread, and isolation of cholera bacilli from a stool sample. Treatment typically involves the compensation of water loss through rehydration by taking Oral Rehydration Solution (ORS) or drinking lots of water or intravenous fluids in case of severe dehydration to counter water loss, administration of antibiotics, and zinc supplements.

Hepatitis Hepatitis A and E viruses present in contaminated water and food or uncooked vegetables results in liver infection (hepatitis) characterized by inflammation of the liver and yellowing of the skin (jaundice). Other symptoms include fever, fatigue, nausea, vomiting, abdominal pain especially near the liver, appetite, weight loss, and yellowing of the skin (jaundice), clay-coloured bowel movements, and dark urine. However, generally, infection is not serious and is resolved within a few days. In some individuals, symptoms may persist for several months. The incubation period is 15–50 days. Diagnosis is by clinical examination for signs of jaundice, and blood test. Supportive care, taking rest, eating homemade and thoroughly cooked food, and having plenty of water help in recovery. Usually, no drug is required. Prevention can be done by using boiled water for drinking or using for cooking purposes and by adopting good hygienic conditions. The best way of prevention is vaccination.

Besides medical treatment, other measures to ease out the symptoms of waterborne diseases are supportive care, taking plenty of water to keep rehydrated, and enough rest to heal the body. Most (Over 95%) of the infections transmitted by contaminated water or food are treatable and can be prevented by adopting the following measures:

- Adopting personal and environmental hygiene
- Employing relatively inexpensive technologies to make the water potable
- Ensure consumption of clean safe water-either potable or filtered using purifying devices for drinking, cooking, or bathing
- Thorough washing & cooking of food
- Practice food safety precautions and avoid consuming stale and unrefrigerated food
- Preserve prepared food from an unclean environment
- Washing hands regularly with soap after using the toilets, coming home from outside, and before and after taking food.
- Avoid ingestion of water from swimming pools
- Avoid public restrooms
- Imparting knowledge to food handlers and consumers regarding hygiene and sanitation
- Immunisation against common waterborne preventable diseases like polio, hepatitis, typhoid, etc.

Challenges

The United Nations adopted 17 development sustainable goals, of which the goal 6 emphasises on the accessibility to safe and affordable water, sanitation and hygiene to everyone by 2030 (Chen & Suga, 2015).

However, the availability of safe and clean water to billions of people across the world is still a challenge. The reason is the increase in population, urbanization, scarcity of water, climate change and surge in water need. Only around 5.8 billion people use "safely managed drinking water services" i.e., the source for drinking water, free from faecal or other contaminants, is located on premises and water is available whenever required. In Spite of the combined efforts of Governments, NGOs, and communities over the years, which has resulted in an increase in the availability of water and sanitation to people, still the efforts need to be sped up by four times to achieve the target of 2030. Though there is a decrease in the number of people using contaminated water from approximately 16.5–8% of the global population (Kahn, 2019), still over 2 billion people are devoid of "safely managed drinking water services". Of these, 282 million people have limited access to safe water i.e., travel for more than 30 minutes to collect the water. The situation is more serious in rural areas where out of ten, eight are lacking good water availability (Chen & Suga, 2015). Globally over 500 million, mostly residing in rural and hard to reach areas, drink contaminated water from unprotected and untreated water resources including ponds, rivers, hand-dug wells, springs, swamps etc. that make them sick and impact their earning and productive time.

Water stressed situations demand increasing reuse of wastewater for agricultural practices. It constitutes around 7% of irrigated land in developing countries which if not properly managed creates health risks (WHO, 2022). Unclean water and unsatisfactory sanitary conditions affect the lives of hundreds of millions of people each year. About one third of the global population (over 2 billion people) live in water-stressed areas and have unavailability of basic sanitation facilities. Most of these people defecate in open areas and fields, which poses a health risk and one of the main contributors of water contamination that causes diseases and deaths, especially of young children below 5 years of age. In 2021, five million children died and one of the leading causes remains diarrhoea. Contaminated water and poor sanitation not only lead to frequent illnesses and death but also impact their health, nutrition, education, and economy.

A number of organizations are working together to end the global crisis of access to safe water and poor sanitation. Guidelines regarding water quality and safe use of wastewater have been produced by international organizations and different programs like safe water projects, and WASH (water access, sanitation, and hygiene) have been implemented to reduce the burden of unsafe water and provide people with clean water (Griffiths, 2008).

The biggest challenge in providing safe accessible drinking water to public is scarcity of funds for effective water treatment and sanitation in bulk, lack of governance and urgency setting despite of known strategy. Other challenges include

population increase, tolerance of pathogens to traditional water treatment, raised level of industrial chemicals in water, quantification of water-related disease, and understanding linkages to the environment. Lack of knowledge about safe sanitation and hygiene among people is again a big challenge. New technologies including use of satellite imaging and recent scientific & mathematical tools, can be employed for studying waterborne diseases. Moreover, educating people and better availability of clean water resources will reduce the illness risk and improve productivity and the economy. A collaborative interdisciplinary approach involving a number of stakeholders including government representatives, community leaders, and health professionals will help in achieving the goal of clean and safe water accessibility to everyone (Griffiths, 2008).

Water Treatment for Safety Concerns

Breakdown of water supplies often constitutes the most significant point of entry for the outbreak of epidemics such as cholera, malaria, typhoid, amoebiasis, and waterborne dysentery. Sewerage, industrial effluents, surface run-off, and a plethora of other anthropogenic activities introduce pathogens into the water as well as alter its physicochemical characteristics (Newton & McClary, 2019). Therefore, efficient treatment of water is of utmost concern for ensuring a safe water supply. Water treatment is a sequential multi-step procedure that removes contaminants and undesirable components from water and renders its quality improvement, intended for a specific end-use like drinking, irrigational use, industrial use or safe recycling to a water body. Following are the major procedures and practices adopted by the municipality for decontamination and optimal recycling of water.

Sedimentation Probably, the most fundamental and ancient technique of water purification is sedimentation (Farhaoui & Derraz, 2016). Due to its ease of operation and minimal cost of implementation, it remains to be the most popular method for the preliminary treatment of water. The process involves the physical settling of impurities by sheer gravitational force or aided by mechanical intervention depending upon the concentration of suspended particles present. In case of low concentration (<1% v/v), particulate impurities can settle down without impacting each other; however, at higher particle concentration, effective sedimentation is impeded by inter-particle interaction. At concentrations exceeding 8% (v/v), no further passive sedimentation can occur. In order to aid the process, many specially designed tanks have been used.

Horizontal Flow Tank These tanks offer the simplest construction and therefore are routinely used to effect sedimentation. The design of these rectangular tanks induces a horizontal flow of water causing vertical separation of particles during the motion. Therefore, the outlet water is mostly freed from suspended particulates. The tank also contains special arrangements to remove and clean the sedimented impuri-

ties from the bottom intermittently. A more improvised version of this basic design is the multi-layer tank which houses several chambers through which water is forced to pass successively. This procedure achieves more effective sedimentation as compared to the basic arrangement.

Radial Flow Tank As the name suggests, radial flow tanks entail a circular design so as to induce a radial movement of water thus affecting sedimentation by centrifugal motion instead of passive displacement by gravitation. This design is also suited ideally for carrying out flocculation and subsequent recirculation.

Settling Tank A specific variant of conventional sedimentation tanks is the settling tank with an inclined construction to aid sedimentation without any mechanical device. Settling tanks are generally huge and deep, containing strategically placed inclined plates at the bottom which can be removed and cleaned periodically for disposal of sediments. The design also accommodates for the flow of water in multiple directions to facilitate the sedimentation process.

Ballasted Sedimentation In cases where the source water quality fluctuates frequently and randomly, a modified methodology of sedimentation termed Ballasted sedimentation or ballasted flocculation is employed (Lapointe & Barbeau, 2016). As the name suggests, the technology is designed to remove recalcitrant flocs onto the ballast (sand) utilizing a high molecular polymer which effectively increases the density of the floc and facilitates its separation from the water.

Floc Blanket Sedimentation An improvised variant of sedimentation tank to take care of flocs is the floc blanket sedimentation. The overall appearance of these tanks resembles an inverted pyramid inside which, a circular motion is achieved to accelerate the formation and deposition of the floc in the form of sludge. However, a major challenge of this type of sedimentation is the requirement of keeping it fluidized all the time by removing the sludge settled at the bottom regularly.

In order to further expedite the process of sedimentation by reducing the time for the process, new generation technologies like Sirofloc® have been invented. Sirofloc® are high magnetite particles in an acidic buffer. During the movement of water through this tank, the intrinsic nature of the magnetite particles results in their self-attraction, floc formation and sedimentation. Finally, the water passes through a radial flow tank where the magnetite particles are collected and reused.

Coagulation and Filtration Coagulation constitutes the most common aided purification strategy of surface water, used for removing particulate matter and turbidity by the addition of iron or aluminium salts which carry a net positive charge in water. These coagulants bind to the negatively charged particulate dirt and bring about their separation from bulk water interphase. The process is also aided by the development of large aggregates of coagulants and dirt. Although the term coagulation is used sometimes synonymously with flocculation, the former involves the neutralization of charge on dirt particles, whereas flocculation essentially means the devel-

opment of larger particulate entities aided by intermolecular association. During water treatment, the coagulant must be added immediately and mixed rapidly with bulk water so as to ensure complete precipitation of dissolved particulates. Coagulation is able to take care of a large number of organic compounds, including Natural Organic Matter (NOM) and Dissolved Organic Carbon (DOC). Excessive DOC imparts an unpleasant taste and odour along with brown coloration.

Filtration is routinely used to effectively remove coagulated SPMs. It is basically a coupled process following coagulation using porous beds. Medium filters such as sand or gravel are often the materials of choice owing to their low cost and effectiveness. A grain of fine sand measures approximately 0.1 mm in diameter, and therefore coagulant-dirt complexes greater than this size are retained in the sand bed. Sand filtration can again be of two types; slow and rapid. The slow method employs "schumtzdecke" -a bacterial biofilm (Ranjan & Prem, 2018) that forms on the topmost layer of sand and degrades the organic matter present in water as it passes through. However, the biofilm layer gets clogged often and has to be cleared periodically. After each cleaning, the filter takes a few days to regenerate the community and the biofilm.

The flow rate of water through a slow sand filtration is limited to 0.1 and 0.3 meters per hour. A faster method is the rapid sand filtration rate which employs either pressure-driven (Paterson's filter) or gravity-driven filtration (Candy's filter) of water (Spellman, 2008). Rapid sand filters also need cleaning on a daily basis by backwashing, a process of reversing the flow of water through the filter bed to remove the accumulated particulates. The released waste generated from backwashing known as "sludge" is either dumped as a landfill or subjected to further secondary treatments according to its composition.

Almost all of the above-listed methods are meant for removal of suspended particulates including organic matter; however, they do not yield water of drinkable quality since these treatments are unable to take care of the microbes (bacteria, viruses and protozoa). Microbes are too small and therefore can easily pass through filters used in water treatment plants. Therefore, drinking water production plants employ physical or chemical agents which can effectively kill the pathogens present in water. Chlorination, Ozonation, and UV treatment are the three industrially used methods for obtaining germ-free water (refer Fig. 14.1).

Chlorination Chlorination is the most widely practised method of chemical disinfection due to its low cost and easy availability. Chlorine can effectively kill most of the waterborne pathogens which are causative agents of typhoid fever, dysentery, cholera and Legionnaires' disease. Chlorine acts by damaging cell membranes which results in leakage of macromolecules from the cell and its eventual death. Basically, in solution, chlorine rapidly hydrolysed into hypochlorous acid and hypochlorite ions which exerts a toxic effect on cells. Therefore, the most common form of addition of chlorine to water is sodium or calcium hypochlorite. The killing potential of any chemical agent is defined in terms of its CT value (Concentration of the reagent times the time taken for complete disinfection). A lower CT value indicates the greater potency of the antimicrobial to kill the cell. The CT value is depen-

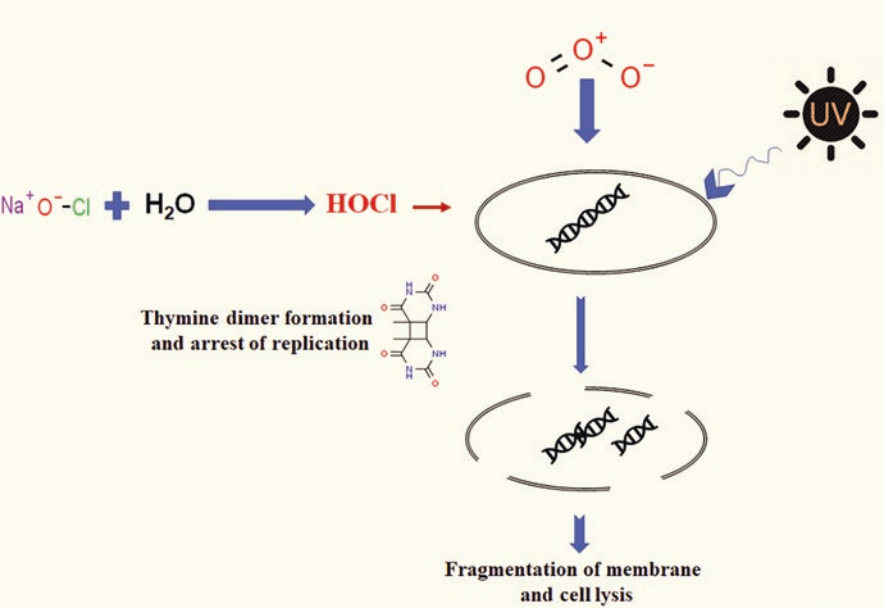

Fig. 14.1 Chlorination, ozonation and UV induced disinfection of water. *Chlorination and ozonation causes membrane rupture whereas UV induced thymine dimer stalls replication and kills the cell*

dent on such as the pH of water and temperature of treatment. Generally, chlorine is added in the final step of treatment in municipal drinking water supplies so as to avoid recontamination. Commercial chlorine sources often contain significant amounts of trichloromethane, a carcinogenic compound known to cause bladder cancer, asthma and cardiac problems. Therefore, it is absolutely mandatory for municipal water supplies to check the amount of chlorine added before discharge of water for consumption. Estimated safe level for human use is considered to be 4 ppm.

A potential disadvantage of using chlorine stems from the fact that it is used up quickly in the bulk water. Therefore, places located at longer distances from the supply source become vulnerable due to loss of effectiveness of chlorine and chances of subsequent re-entry of pathogens. Therefore, a substitute in the form of chloramination has been in vogue to ensure more effective delivery of safe water. Monochloramine, often called simply chloramine (NH2Cl), is a stable alternative to the use of chlorine which has an even broader spectrum of action against microbes (Marchesi et al., 2020). It is especially more effective when generated in situ by downstream addition of ammonia in the supply pipeline. However, it acts slowly as compared to chlorine and hence a higher CT value and additionally imparts a pungent smell to the water unique to ammonia. Tolerable chloramine level in drinking water supply is also 4 ppm like chlorine. Often, water supplies use a combination of chlorine gas, sodium hypochlorite, and ammonia at different entry points in a long distribution system to preserve the safety of the water.

Ozonation The ozone layer of the atmosphere acts as a natural shield against harmful UV-B and UV-C radiation. However, its powerful oxidizing action (Eo = 2.07 V) forms the basis of its strong potential to decontaminate water. In water, hydroxide ions and other solutes catalyze the decomposition of ozone leading to the generation of peroxy and hydroxyl free radicals. These can catalyse further decomposition of ozone thus leading to the generation of an avalanche of radicals that bleaches and disintegrates the membrane components leading to protoplasmic oxidation and subsequent cell death. The extent of killing depends on the target organism's susceptibility, time of contact, and the concentration of free radicals generated. The success of disinfection depends on several key factors including target organism susceptibility, duration of contact, and ozone density. Besides its antimicrobial action, ozone also leads to the oxidation of dissolved iron, manganese, and copper salts thus resulting in their precipitation and subsequent removal by filtration.

Ozone generation in wastewater treatment plants is generally carried out by the installation of an alternating current facility with a high potential (6–20 kV) that discharges in a dielectric gap saturated with oxygen. This leads to *in situ* production of ozone which is immediately fed into the water tank.

UV Radiation Ultraviolet (UV) radiation belongs to the broad spectrum of electromagnetic radiation and is classified into four sub-regions according to the wavelength; the highest energy radiation is constituted by the vacuum UV region (200–100 nm) followed by UV-C (280–200 nm), UV-B (315–280 nm) and UV-A (380–315 nm). The germicidal property mostly comes from the UV-C zone since nitrogenous bases of nucleic acids absorb strongly at 257–265 nm leading to the formation of a plethora of photoproducts including thymine dimers and cytosine hydrates (Rastogi et al., 2010). The formation of these structures causes bulging of the double helix and thus arrests bacterial replication. The efficiency of radiation is expressed as the UV dose or as the energy per unit area falling upon a surface, expressed as the product of the intensity of radiation (I) and exposure time (T).

Water treatment plants as well as domestic appliances rely on a series of mercury vapour lamps as the most efficient and dependable source of UV-C radiation. The intensity of radiation is determined by the density of mercury atoms in the UV lamp which in turn is directly proportional to the vapour pressure of mercury. Accordingly, three different types of UV lamps are used in water treatment; low-pressure (LP), low-pressure high output (LPHO), and medium-pressure (MP) lamps. Although low pressure lamps emit the weakest intensity of UV in terms of power output, they produce a constant monochromatic radiation at around 254 nm. The quality of the water to be treated is a major determining factor during the installation of a UV reactor facility; the presence of too much dissolved organic matter and particulate entities impedes the penetration of the radiation by shielding the microbes. Therefore, for the treatment of industrial effluents, a series of high vapour pressure lamps are to be employed. Additionally, the rate of flow of water also constitutes an important factor since it governs the time of contact of water with the fixed UV source.

Copper-Silver Ionization A relatively less popular method of disinfecting potable water owing to its high cost of installation and maintenance is the use of the oligodynamic action of metal to kill pathogens. Copper-silver electrodes are one of the few devices that can kill *Legionella pneumophila*, a gram-negative bacterium responsible for Legionnaires' disease, an acute and often fatal form of pneumonia. The method is based on the dispersion of positively charged copper and silver ions into water and their subsequent binding to the negative charges on bacterial cell walls. Eventually, membranes are disintegrated through metal ion binding induced denaturation of membrane proteins. Copper and silver ions also have the potential to break biofilms over a long period of time (30–45 days).

The various physical and chemical methods of water treatment discussed above have their own specific virtues as well as demerits, as summed up in Table 14.3.

Strategies to Improve Water Quality in Food Sector

Water is probably the most indispensable component of any food processing/preservation industry. Its usage involves either direct contact with the food, or with the surfaces to which food comes in direct contact (containers etc.) or else as a processing aid (steam etc.) Therefore, it is equally important both with respect to product as well as production process. While setting up an effective water quality assessment system in a food industry, proper monitoring of the following aspects can ensure sustained supply of safe water for the production process.

Identifying the Source of Water The very first objective while setting up a network of water supply for the food processing/preservation industry is to identify the source of water as well as its quality. The best source is generally the municipal water supply since it is often treated to an adequate extent. However, if the water is collected from a natural water body such as river or lake, the industry must ensure adequate pre-treatment operations including chlorination/ozonation/UV sterilisation before it is supplied for its intended use.

Gathering Complete Information About the Water Supply System The industry should prepare a blueprint of the water supply network from the source to delivery point. It should also make a careful assessment in terms of volume needed at various delivery points and thereby decide the water pressure needed. Lower than adequate water pressure is ideal for the re-entry of pathogens in the main supply line and therefore detrimental to the industry. It is also equally important to correctly assess the volume of wastewater produced and arrange for easy drainage of the same to the post-production water treatment plant before releasing back into the water body. The pipelines used in the supply network should be non-corrosive and of the highest grade so as to ensure that no odour or taste is imparted to the water. They should also be resistant to intermittent cleansing processes.

Table 14.3 A comparative account of the merits and demerits associated with various water treatment strategies

Treatment method	Advantage	Disadvantage	Reference
Sedimentation	Most eco-friendly process since no chemical is involved. Inexpensive process requiring minimal set-up.	Takes care of only larger particulate entities. Does not disinfect water	Falco et al. (2020)
Coagulation	Significantly reduces the time for settling of particles in wastewater. Also facilitates settling of colloidal particles and mineral contaminants.	Sludge requires monitoring and toxicity testing before testing due to addition of chemicals.	Jiang (2015)
Chlorination	Strong oxidising agent and hence can exert its effect even at low concentrations	Optimal action confined within short distances from the point of application. Can disrupt biofilm only above 50 ppm which poses serious health hazards.	Nielsen et al. (2022)
Chloramination	Better penetration power over biofilms as compared to chlorination. Has longer effectivity than chlorination when addition of ammonia is downstream from the initial chlorine forming *in situ* monochloramine	Much lesser oxidising potential as compared to chlorination.	Hossain et al. (2022)
Ozonation	Strong oxidizing potential arising out of continuous exponential formation of free radicals. Effectiveness is higher than chlorination in killing viruses and bacteria with a shorter contact time. Inhibits biofilm formation	Tends to get consumed quickly through the neutralisation of free radicals by reaction with dissolved organic matter. Grossly ineffective against spore forming bacteria, some viruses, and cysts at low doses. Not cost effective for water with high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)	Wei et al. (2017), Panebianco et al. (2022)

(continued)

Table 14.3 (continued)

Treatment method	Advantage	Disadvantage	Reference
UV radiation	Strong antibacterial potential with minimal time of exposure. Doesn't impart taste or odour to water. Causes inactivation of *Cryptosporidium sp.* and *Giardia sp.*	Cannot penetrate biofilms. Performance is greatly affected by quality of water.	Zewde et al. (2020), Adeyemo et al. (2019)
Copper silver ionisation	Can break down and inhibit biofilm. Effective against *Legionella*	High cost of maintenance	Wu et al. (2019), Cachafeiro et al. (2007), Triantafyllidou et al. (2016)

Maintenance and Management of Statutory and Regulatory Mandates The governing body should procure and retain all the statutory and regulatory requirements to comply with the water usage policy according to the regional/country and guidelines and also have thorough knowledge about the corresponding international regulations and policies.

Maintenance of the Plant A separate maintenance unit should be set up to look after the water supply and wastewater disposal system of the industry. This includes pumps at various points of the supply line, water treatment equipment, consumables employed in water treatment, back-flow prevention devices, hoses, taps, and quality monitoring devices. The performance of each of the above should be assessed at least once every year and corrective measures for replacing faulty/non-working devices should be taken immediately.

Risk Assessment and Hazard Analysis A specialist team should be appointed to carry out a thorough research about the surroundings of the water source (for e.g., pH, water holding capacity, salinity and gross microbial profiling); preferably, this analysis should be conducted several times accounting for seasonal variations as well as anthropogenic activities. All components of hazard analysis should be carried out according to Hazard Analysis and Critical Control Point (HACCP) regulations. HACCP is a set of rules which identifies and efficiently manages various aspects of safety issues in a food industry including biological, chemical and physical hazards starting from dealing and/or production of raw materials to manufacturing, distribution and consumption of finished products (Agyei-Baffour et al., 2013).

Methodologies for Detection of Contaminants in Water

Frequent detection of biological and non-biological contaminants in water supply networks is integral to the safety of the food industry. In addition to the traditional practices for assuring the safety of supplied water to the food industries, new generation innovations and tools for state-of-the-art continuous monitoring of water quality have been invented which can revolutionize the management of water supply.

Detection of Microbial Contaminants Multiple tube fermentation (MTF) technique is the most widely used and economical method to gain an overall assessment of the presence of microbes in test water samples meant for use in the food industry (Umar et al., 2019). It involves three stages, the presumptive test which is based on the ability of lactose fermenters to utilize lactose broth with simultaneous evolution of gas and drop in pH of the medium due to the production of acids as end products. This is succeeded by the confirmed test which eliminates the false positive gas production by non-coliforms by growing them on an Eosin-Methylene Blue agar medium. The presence of methylene blue ensures that only gram-negative organisms are grown thus eliminating the chances of any false positive detection of coliforms. Finally, the colonies appearing on EMB plates are confirmed through gram staining and morphology analysis. A modified version of the presumptive test includes the employment of a group of test tubes with three different concentrations of lactose broth and gives a rough estimation of the number of coliforms/ml of the test water sample (Most Probable Number) based on statistical analysis. Apart from the MTF method, membrane filtration is another popular method for enumerating the number of microbes in a water sample. The method includes the entrapment of the microbe onto the membrane surface as water is forced through the membrane; subsequently, the membrane pad is placed onto the surface of a selective medium (agar) for detection and enumeration of a specific pathogen.

Although E. coli is accepted as the standard indicator organism for the presence of pathogenic bacteria in a water sample, it cannot predict the presence of viral pathogens. Therefore, the use of viral indicator organisms such as Pepper mild mottle virus (PMMoV), an abundant RNA virus of human faeces, is now being increasingly and successfully used along with *E. coli* (Kitajima et al., 2018). PMMoV is a plant virus of the genus Tobamovirus in the family Virgaviridae which probably landed in the human gut via the consumption of peppers. It is also an equally abundant virus in human faeces.

Apart from the quantitative techniques, an integral part of safe water analysis also requires the detection of specific pathogens that might occur in low numbers and therefore escape the standard microbiological detection procedures, Real-time PCR (a.k.a. qPCR) and Fluorescence in situ Hybridisation (FISH) are two vastly employed methods to achieve this purpose. qPCR was successfully used to detect the pathogenic strain O157:H7 of E. coli in drinking water using oligonucleotide probes. Usually, FISH employs a 15–30 nts long fluorophore-labeled probe which

can bind to specific mRNAs from microbes. A particular study has reported the successful detection and quantification of Cytophaga-Flavobacterium along with β-Proteobacteria in a river of urban area (Bouvier & Del Giorgio, 2003).

Detection of Non-biological Contaminants Many toxic contaminants of non-biological origin such as heavy metals (Pb, As, Hg, Cd), radioactive materials (plutonium and uranium), oxides of metallic nanoparticles (TiO_2, ZnO, and CeO_2) as well as quantum dots (ZnS) can pose a serious threat to community health if left undetected. Therefore, both old and traditional methods such as capillary electrophoresis, gel electrophoresis, and mass spectroscopy, as well as modern techniques such as the use of nanosensors, are frequently used in water quality monitoring plants. Nanosensors, which are built of nanomaterials along with a recognition element, and a signal transduction element, are optimally suited for *in situ* detection of trace amounts of contaminants. In addition, bioconjugated quantum dots or inorganic semiconductor nanocrystals (Kaur et al., 2019), surface conjugated with biological macromolecules such as specific antibodies against membrane receptors can also efficiently detect pathogens.

Conclusion

Water has the most integral role to play in ensuring the safety and success of a food production/processing unit. Therefore, frequent and critical monitoring of water quality is of paramount importance. The importance and value of water has been grossly underrated globally. We are nearing a point where access to safe drinking water will be limited and restricted. It is imperative that the crisis will also hit those industries which are heavily dependent on water. Under the circumstances, proper management of the water supply network as well as proper disposal of wastewater after adequate treatment are absolutely indispensable for not only ensuring the success of that industry but also safeguarding the health and interest of all those working in the industry or living within its short radius. On the other hand, much as water is an elixir of life, it can equally well be the harbinger of several epidemics if not managed properly. It is the carrier of a plethora of pathogenic microbes including bacteria, viruses, and protozoa. In order to effectively combat and mitigate waterborne diseases, the role of man and machine become equally important. Most modern food industries have enough skilled personnel as well as advanced, state-of-the-art treatment and detection devices to ensure a sustained, safe supply of water to the industry as well as thwart any sudden adverse conditions such as drought or flooding. Therefore, understandably enough, the water footprint across the globe has silently crept up. Probably, the biggest challenges to all food industries in the coming decade will be a) ensuring a steady supply of water and b) effectively decontaminating the supply line over long periods of time.

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Chapter 15
Challenges in Environmental Biotechnology

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Introduction

Biotechnology is a broad field that includes the change and improvement of specific qualities of plants, animals, and microorganisms to facilitate new commodities, strategies, and organic entities that are planned to work on human well-being. It is a versatile field that plays a significant role in many areas, including agriculture, genetic technology, medicine, food technology, environmental biotechnology, etc. Proficient utilization of biotechnology gives social, financial, and environmental advantages by utilizing some cutting-edge biotechnological techniques like gene modification, chimeric DNA technology, and life sciences to produce a product that is beneficial for humans and does not possess destructive effects on the environment as well (Gavrilescu, 2010). Biotechnology is broadly classified into five main categories: Plant Biotechnology, Animal Biotechnology, Microbial or industrial biotechnology, Health or Medicinal biotechnology, and Environmental Biotechnology (Bach & Bich Thuy, 2019). Out of these, the environment is one of the significant regions where the most incredible exploration occurs.

Environmental biotechnology is a branch of biotechnology that deals with the usage of certain methods or processes to minimize the hazards occurring from anthropogenic activities at the domestic or industrial level that threaten our environment. It generally involves the check of decontamination of environmental components (such as air, water, soil), waste minimization occurring from industries (food, petroleum, pharmaceutical, chemical, oil, and gas industries) for environment

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protection or biomonitoring and the preservation of the non-renewable natural resources and energy contributing towards sustainability. It also includes the human practices that are employed during the production of a product, generally inducing environmental hazards which lead to the degradation of our natural habitat. Moreover, some treatments are employed during the pre-and post-manufacturing of products to minimize the degradation of the natural biosphere. Pretreatment of raw material is necessary for cleaner production and fewer generation of contaminants (occurring during the production process).

Along with reducing the production of harmful substances, environmental biotechnology also leads to sustainability. It considers energy efficiency while developing and applying new technologies to minimize wastage (causing toxicity) and further contamination of our natural resources. Cleaner production involves less wastage, high yield, and a limited number of steps during production, environment friendly and safer for consumption (EIBE, 2000; Gavrilescu & Chisti, 2005). Primary sources from where degradation is usually observed are Industries, Transportation, and Agricultural and Domestic practices (Fig. 15.1). Production of Toxic/hazardous waste, toxic gases such as nitrogen dioxide, Sulphur dioxide, carbon dioxide, and chemicals from the above sources give rise to environmental degradation (Elehinafe et al., 2022). However, due to rapid industrialization and modernization, there are many implications that we are facing in environmental biotechnology, which are briefly discussed in the chapter. This chapter focuses on different challenges that are faced in environmental biotechnology. Challenges differ depending on the type of process that is done for treating waste and minimizing hazards. There are numerous ways to treat wastes that are discussed in the chapter (Fig. 15.2).

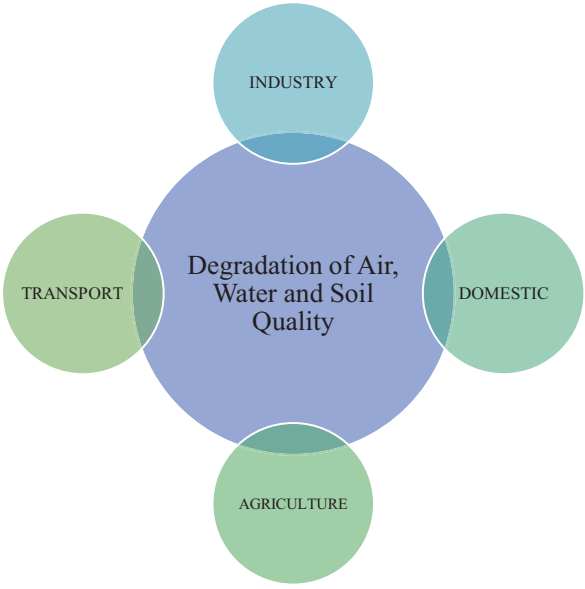


Fig. 15.1 Sources of degradation

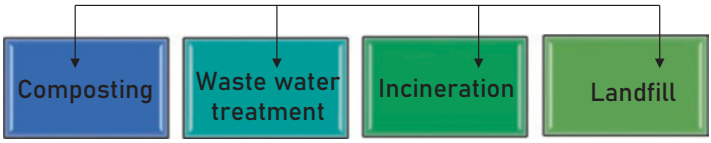


Fig. 15.2 Processes of waste disposal; Common challenges that can be applied to all situations

Composting

Composting is a great way to reduce the amount of waste, i.e., converting degradable organic waste into steady products with the help of bacteria that produce and help us to improve our environment. Several challenges can come with composting, but with the right solutions, composting can be a beneficial and successful process Ayilara et al., (2020). The benefits of composting include:

- Reduce the amount of waste that ends up in landfill
- Improve soil fertility
- Reduce the need for fertilizers, pesticides, and herbicides

The restriction incorporates identifying pathogens, inferior supplement level, and time duration of the process.

Process of Composting

The fundamental part liable for biodegradation and fertilizing the soil is the modification or interaction by the microbial community. Blended microorganisms achieve it. The multitude of microorganisms known to exist, bacteria and fungi have the most noteworthy populaces while fertilizing the soil. Two unique gatherings of microorganisms partake in fertilizing the soil, the primary gathering incorporates mesophilic organisms, and the next gathering is thermophilic microorganisms. These include actinomycetes, molds & yeast. The various mesophilic and thermophilic stages are significant in fertilizing the soil cycle. Mesophyll is the initial step when the temperature is between 20 and 40 °C. Then follows the thermophilic stage medium temperature stage. Generally, dynamic disintegration happens in the thermophilic period (40–70 °C). Mesophilic organisms entities are obliterated or inert during this period, and thermophilic as well as intensity-safe microbes and actinomycetes. The secondary step is considered a curing step in which compost usually matures.

Challenges in Composting

Figure 15.3 shows the challenges that generally take place during composting.

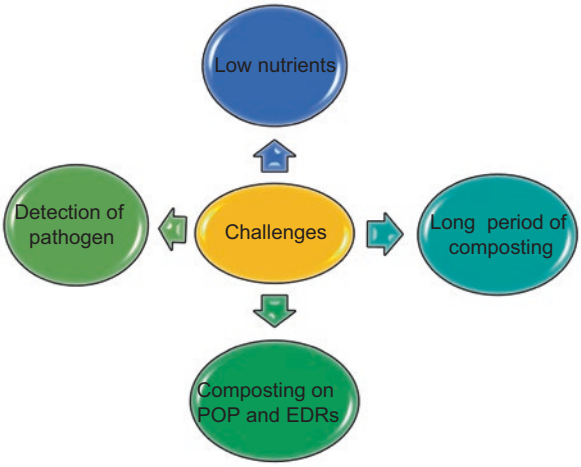


Fig. 15.3 Challenges during the process of composting

Long Period of Composting

Farming debris inhabits possess an enormous level of farmstead's waste. Waste carries resistant blends and common nutrients that drive their composting challenging. Some components in waste, such as lignin, polyphenol, cutin, and suberin content, enhance the composting time. Lignin is exceptionally convoluted to compost due to its resistant qualities; favourably, phenolic blends bring a more elongated period to compost because of their complicated chemical configuration (Lewis & Brown, 2010). Co-composting is the acquisition of materials together during composting to convey around a prime ratio of C: N for devising a fertilizer attribute. Carbon and Nitrogen proportion is inversely associated with the span of composting that represents the loftier the proportion of carbon and nitrogen is also difficult for bacteria to decompose and it takes longer for compost and vice-versa. For the reduction of C: N, activators are used, which contains microorganism that helps in degrading the raw material. For example, cow dung, sewage, pig dung etc. (Bhatti et al., 2017; Cofie et al., 2016; Arumugam et al., 2018; Iewkittayakorn et al., 2018).

Nutritive Value

It is essential to check the nutrient value and type of compost because they boost produce output because of the nutrient's existence. Similarly, some nutrients do not cause any growth of the plants. Also, the nutrient-rich substrate should be added to improve the nutritive value (Sharma et al., 2017; Lawal & Babalola, 2014; Masowa et al., 2018).

Studies found that nitrogen, phosphorous, and potassium content should be as follows.

(a) Nitrogen – >1%
(b) Phosphorous – >1.5%
(c) Potassium – >1.5%
(d) Other micronutrients (calcium, zinc, copper) – 0.01–0.05%

Detection of Pathogens

Composts ought to be appropriately analyzed for microbial and chemical constituents for well-being (Alvarenga et al., 2015). Activators are utilized for diminishing the duration of composting the soil that can contain a few microorganisms as they are brought from cow dung, pig dung and so on. Various illness-causing microorganisms such as E. Coli, Salmonella & Thermoactinomyces species cause sensitivity illness of the respiratory framework and severe disorders among farmers. (Chen et al., 2018; Wu et al., 2015; Epelde et al., 2018).

Organic Pollutants and Disruptors

Persistent Organic Pollutants (POPs) and Endocrine Disruptors (EDRs) are tainted chemicals fragment in soil and water etc., which are challenging to detoriate. These are comprised of aromatic hydrocarbons and nonylphenols, which are destructive to well-being (Dodgen et al., 2013). Contamination can occur by chemicals absorbed by soil and water. Consuming these plants can lead to the bioaccumulation of EDRs in humans. Getting rid of these chemicals is not easy but composting has offered a great influence in annihilating these hazards. A detailed approach should enhance farming and ecologically legitimate. The existence of microbes in compost aid to immerse POPs. Thus, bioavailability of POPs is very problematic for their immersion. (Brambilla et al., 2016; Braunig et al., 2017; Luo et al., 2018).

Effluent Treatment

Wastewater treatment is done to discard contaminants from water to meet quality standards. There are different processes for treating wastewater (Quach-Cu et al., 2018) that can be grouped as in Fig. 15.4 (Fig. 15.5).

Energy Consumption Energy necessities quintessential to employing an effluent treatment is a challenging chore. The wastewater filtration approach eradicates a vast share of electricity yearly Hosomi (2016). In city effluent treatment, the best level of solidarity is utilized in the secondary treatment commonly in the spectrum of 50% of factory use.

The issue can be overwhelmed by making modifications in natural treatment processes for instance involving membrane innovation in the air circulation process.

WASTE WATER TREATMENT		
PRIMARY TREATMENT It consist of removing large particles and suspended solids by using sedimentation, screening, etc processes	SECONDARY TREATMENT It consists of removing colloidal and dissolved matter by using microorganism which is not possible to remove by primary method.	TERTIARY TREATMENT It involves removal of those materials that are carried out during secondary treatment by using microstraining, chemical coagulation, settling, etc.

Fig. 15.4 Process involved in wastewater treatment

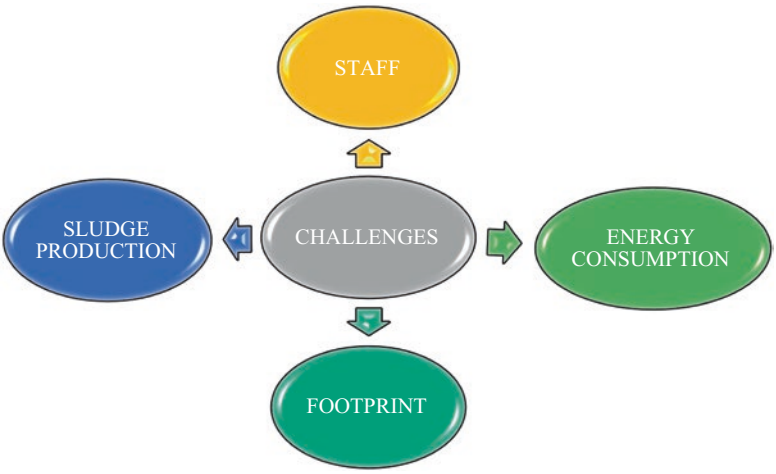


Fig. 15.5 Major challenges faced during wastewater treatment

Additionally, greener approaches are in the spotlight for diminishing energy utilization and diminishing the burden on the power matrix.

Staff Like different enterprises, locating a certified factory administrators has evolved a challenge. Administrators should be appropriately prepared and ensured. There could be as of now not more than adequate faculty to go around. They should be available 24/7 and responsible for supervising everything from leakage of pipes to electrical equipment. This work will become mainly annoying during changes in season and influent.

Discovering ways to deal with incorporating programmed techniques & distant activity strategies might also help. These headways will aid limit the dependence on staff that might have arisen. It would diminish the necessity of administrator engagement.

Fig. 15.6 Steps for treatment of sludge

Sludge Production During wastewater treatment sludge is generated. Fundamentally during treatment, leftover is reprocessed and the wasted sludge is very challenging to dispose. It represents about half the cost of the treatment plant. It mainly consists of water and volume reduction is used for economic disposal. To diminish the dampness, sludge could be stabilized so that the biological action is reduced (Rittmann, 2010). The sludge can be treated in the following sequence (Fig. 15.6).

By following these steps, the sludge can be treated effectively, the microbial load can be reduced and the cost can be minimized.

Footprint Wastewater treatment is employed for eliminating contaminants or organic matter and makes it acceptable to use. They leave their footprint during the treatment process or in layman's language we can say that the organic matter that is removed during treatment is to be used or disposed of safely. Green technology can help to reduce the environmental footprint that is left behind. Also, advanced technologies are used like increasing the biomass concentration, and adding media to increase the amount of biomass per unit volume (Sikosana et al., 2019).

Incineration

The solid waste disposable method involves the burning of waste material occurring from industrial, agricultural and household activities at high temperatures and produces energy which is usually termed incineration. It involves the combustion of refuse which is a very traditional method to decompose solid waste but along with waste management, it comprises many implications for the environment. Incineration is the main cause of air pollution as during burning of refuse produces many harmful greenhouse gases and contributes to ozone layer depletion (Astrup et al., 2009). It results in the reduction of 80–90% of solid waste by volume. Incineration is generally carried out in plants by using incinerators. When some of the waste material is burned it also results in the production of more hazardous materials such as toxins (mercury, dioxins and furans), CO_2 and NO_2 which results in the degradation of our natural biosphere (Lopes et al., 2015).

Process of Incineration

Incineration is carried out by following some procedures (Fig. 15.7). This process of waste disposal is initiated by storage of waste in the loading area of the plant where feed is prepared or pretreatment is given to the feed before incineration until the combustion of certain materials is reached. After incineration is done at a high temperature carried out by incinerators, energy is recuperated in the terms of heat to be further used in steam production. During incineration, many harmful gases and pollutants are released and to control or reduce that, an air pollution control system is a general introduction. However, these control systems do not remove all the hazardous effluents and hence some emissions produced are released from a certain stack height. During incineration and working of the air pollution control system, ash is produced which is also disposed of later (Vehlow, 2015) (Fig. 15.8).

Air Pollution The production of toxic gases and chemicals that are released into the air results in the degradation of its quality and became a major parameter in contributing to ozone layer depletion. Many particulate pollutants are produced during incineration such as particulate matter, lead, and mercury and along with fly ash coming from the stack leads to contamination of air.

Toxicity of Ash The ash that is produced during the combustion of certain waste particles occurring during incineration is generally collected and disposed of on the ground and in open landfills. The ash possesses many heavy metals such as cad-

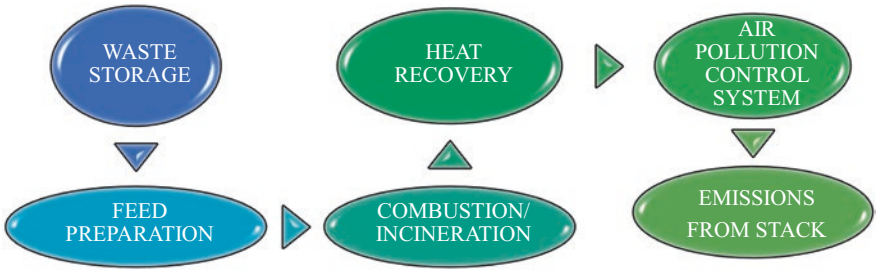


Fig. 15.7 Procedure of incineration (Moharir et al., 2019)

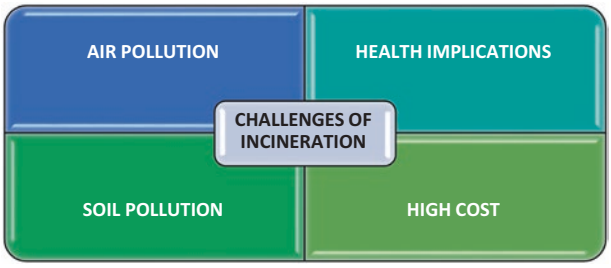


Fig. 15.8 challenges of incineration

mium (Cd), lead (Pb), arsenic (As) and chromium (Cr) which eventually degrades soil quality and is also harmful to humans (Wuana & Okieimen, 2014). The crops produced from this soil can direct to numerous adverse impacts on well-being. Tainted contaminants in ash contain toxins that are present in concentrated form.

Landfilling Incineration is generally done to minimize or reduce solid waste by volume and weight but some materials tend to have non-combustible nature and hence can't be incinerated. As these materials are not incinerated, they are openly dumped into landfills which leads to soil contamination as they have many soil degradation properties.

Short-Term Procedure Incineration is a waste disposable method but it is a short-term controlling procedure. By performing incineration, we can minimize the solid waste by volume and weight but it results in the production of more harmful and toxic substances which are more hazardous to our environment than solid waste.

Health Implications Along with waste minimization, incineration also leads to many chronic diseases which negatively impact human health and well-being. The particulate matter occurring from this procedure causes different lung and heart diseases like lung cancer, heart attack etc. (Allsopp et al., 2001). Along with cardiovascular and respiratory illnesses, it also directs to neurological disorders, which arise from the breakdown of heavy metals such as Cd and Pb present in ash. During this, toxic chemicals are also released such as PFAs and dioxins which are principal components for causing cancer and other deadly health problems.

High Cost Incineration is an expensive procedure to be carried out as the cost of the incinerator and other components used during incineration requires high capital investment in the waste treatment plant.

Landfill

The site or area used for the disposal of solid waste coming from residential areas and industries is termed landfilling. It is the biological method of waste treatment which involves dumping solid waste, garbage or refuse arising from domestic chores and industrial operations. Furthermore, this waste management process is initiated by the collection of refuse from residential areas (non-hazardous) and from industries (hazardous) and is dumped into large open pits where this waste is covered by a thick layer of soil and left for decomposition. This method is considered as traditional and widely accepted approach for waste management. Many harmful impacts can be observed on the natural amenities of our planet. Based on waste origin landfill operation is divided into four categories. Municipal Solid Waste (MSW) Landfills, Industrial Waste Landfills, Hazardous Waste Landfills, Green Waste Landfills (Fig. 15.9). Trash (kitchen trash, tissues, cardboard boxes) arising from household activities and collected by municipal authorities is termed as MSW

landfill. Refuse arising from industries such as concrete, wood, metal, glass etc. comes under the industrial waste landfills. The hazardous materials that are detrimental to the surroundings come under the category of hazardous waste landfills. Hazardous waste being a threat to the environment is not treated or decomposed instead they are collected on the site. The biodegradable or organic debris, food leftovers, plant residues are dumped into landfills falls under the category of green waste landfills.

Challenges Faced during Landfill

When waste management is executed by landfill operations there are certain implications involved during the practicing of this method (Fig. 15.10). They are briefly discussed below:

Fig. 15.9 Types of landfills

Fig. 15.10 Challenges occur during landfill

Air Pollution Decomposition of organic waste leads to generation of harmful gases which are emitted from landfills. Methane and Carbon dioxide contribute to about 90% of total gas production and the remaining portion consists of nitrogen, oxygen, ammonia, sulfides, hydrogen and various other gases. Landfills are the third largest source of methane production which is the main cause of climate change. Due to the emission of these toxic gases, air quality is severely impacted and hence its quality deteriorates.

Groundwater Pollution When rainwater comes in contact with the organic waste (retaining elevated engagements of organic pollutants, heavy metals, poisonous chemicals and inorganic compounds) that is being decomposed by the action of certain bacteria, the contaminated water penetrates the ground and leads to the degradation of the quality of groundwater. Leachate negatively impacts aquatic life and studies have shown that landfills have 82% of leaks globally.

Soil Pollution At the point when waste is unloaded into landfills, it corrupts the nature of soil because of the presence of a lot of poisonous synthetic substances. The penetration of leachate from soil to underground water also results in the degradation of the upper of the soil and makes it unusable for agricultural usage.

Health Implications Toxic gases arising from landfills lead to many respiratory and cardiovascular diseases such as asthma, lung cancer, and heart attack (due to blockage in the arteries). People living near these dumping sites or landfills are also at a major risk of developing birth defects and high mortality rates along with the above-mentioned chronic diseases. As it also degrades groundwater quality and if this contaminated groundwater is consumed it may lead to several deadly health implications.

Economic Cost When solid waste is dumped into the engineered pits, some of the non-degradable materials are sent for recycling operations and the management of these procedures is quite expensive because they require high amounts of initial investment. Along with these large amounts of taxes are also included to ensure regulatory compliance with the set standards.

Landfill Fires Harmful gases like methane arising from these landfills are highly flammable and if it gets to fire, they can cause a fire in the whole dumping site which could cause air pollution. Also, if not controlled it may cause severe damage to the habitat or surroundings of the population living nearby the dumping sites.

Bioremediation

Bioremediation can be characterized as a characteristic or human-controlled process that involves natural catalysts for activity on contaminations, in this manner helping and taking out ecological impurities that are available in wastewater, soil, and so on.

1900 – Municipal wastewater treatment started.
1950 – Industrial treatment of wastewater – deploy different microbial cultures and microorganisms in the wastewater treatment process.
1952 – Microbial infallibility hypothesis- for any chemical or organic compound there is one organism that can degrade or can be used as a biological catalyst.
1970 – Bioremediation of gasoline-contaminated aquifer- groundwater system where gasoline contamination was reported, bioremediation is applied over there.
1980 – Importance of biogeochemical process in bioremediation – it is not microbes that are of concern but it is biogeochemical and also the geochemical processes that are important as they indirectly or directly play a role in controlling bioremediation.
1990 – Hybrid methodologies 2000-In situ bioremediation and checked regular weakening are broadly acknowledged as practical cleanup options. (Fig. 15.11).

By using some techniques, environmental dangers and hazards brought on by accumulated toxic substances, can be reduced or drawn out (Fig. 15.12).

Using approaches for pollution prevention and control, biotechnology can be accustomed to (bio)treat/(bio)remediate pollution. By employing microorganism-based approaches to degrade or transform toxin into slighter precarious forms. It is comprehended by the US Environmental Protection Agency (USEPA) as "a managed or spontaneous approach that remediates or eliminates environmental pollution" (USEPA, 1994).

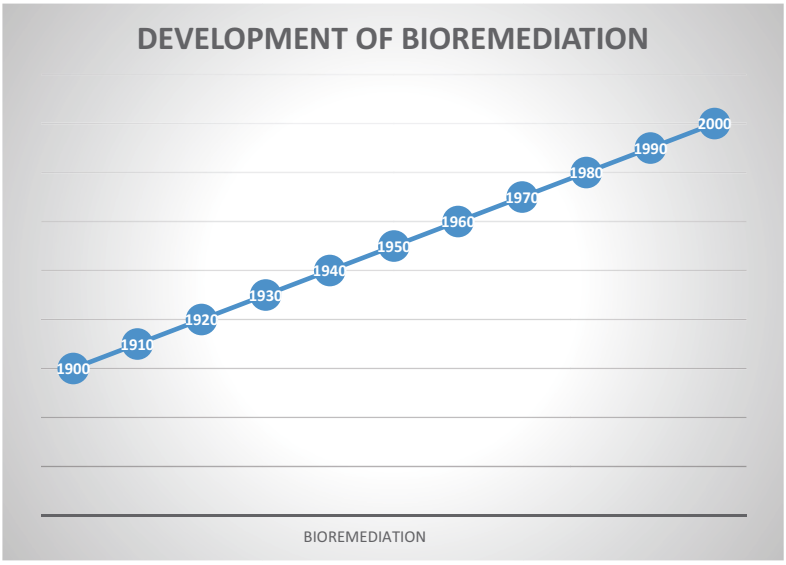


Fig. 15.11 Process development of bioremediation

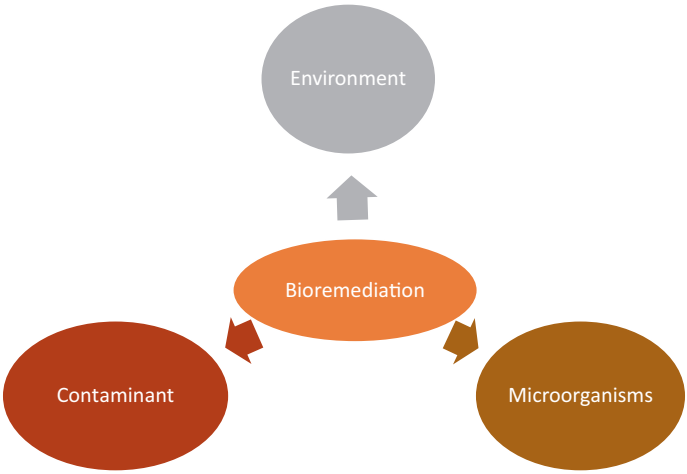


Fig. 15.12 Factors influencing bioremediation

At the point when pollution in natural media should be taken out, debased, or detoxified, procedures like bioremediation and biotreatment are regularly utilized. It further branched into four processes which are as follows (Asante-Duah, 1996; Khan et al., 2004; Doble & Kumar, 2005; Gavrilescu, 2006)

(i) Extraction: The method for removing contaminants or contaminated media from a location without separating them from the host medium
(ii) Separation is a method for removing contaminants out of the host medium.
(iii) Destruction/Degradation: a procedure that eliminates or neutralizes the contamination using chemical or biological means to generate less dangerous molecules.
(iv) Immobilization: Method thwarts or halts contaminant's superficial and subsurface movement (Watson, 1999; Khan et al., 2004; Gavrilescu, 2006)

Environmental biotechnology may be thought of as having a potential use for all living forms., however, they are existing naturally or through intentional introduction. Several of these species can degrade materials. Due to their discovery in hostile conditions, some of the most dangerous and resistant compounds where their shape and metabolic capacity are impacted by their need to survive.

Protozoa, algae, and plants are capable of absorbing nitrogen, phosphorus, and Sulphur, as well as many other minerals and metals from the environment.

Degradation of complex molecules can be achieved by bacteria and fungi & the resultant outcomes they generate are characteristically harmless.

Bioremediation frequently relies on bacteria' innate capacities to enhance their metabolism and maximize enzyme activity.

Commonly, the ideal temperature range for ensuring the best is 20–30 °C, with a pH of 6.5–7.5 or 5.9–9.0. For a specific sort of defilement and natural compartment, the required remediation objectives, how much time are accessible, and different

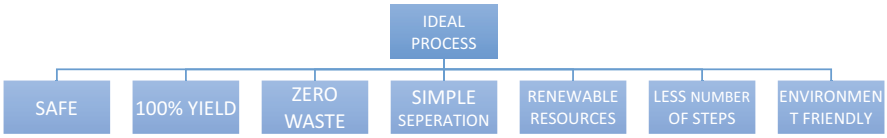


Fig. 15.13 Factors considered in an ideal process

elements like nourishment accessibility, oxygenation, and the presence of extra inhibitory toxins are vital. In light of a careful beginning study and hazard evaluation, the decision of a specific remediation approach might include non-planned arrangements or a designing one.

Despite the fact that bioremediation methodologies have been created for both in situ and ex-situ treatment, (EIBE, 2000; Sasikumar & Papinazath, 2003; Gavrilescu, 2005; Gavrilescu & Chisti, 2005) bioremediation advances actually bring different advantages, including as:

– price reduction related towards competing knowhows for operations.
– Little spot disruption.
– minimal investment expenses the annihilation of toxins & inhibition of distributing the issue elsewhere.
– taking advantage of how other technologies interact with it.

In several significant environmental technologies, such as activated sludge or biofilm in wastewater treatment, microorganisms can exist as independent individuals or as groups in mixed cultures (consortia) (Gavrilescu & Macoveanu, 1999; Gavrilescu & Macoveanu, 2000; Tchobanoglous et al., 1991). Microbial community structures in activated sludges, which are made up of activated sludge flocs and contain a variety of microorganism kinds, are perhaps of the main basic component in the plan of natural wastewater treatment frameworks (Fig. 15.13). (Wagner & Amann, 1997).

Oxygenations of a microbe-rich setting, purification, solid-to-gas conversion, or removal of toxins are approximately of ways that plants contribute to environmental cleaning.

The possibility that all organic entities could eliminate poisons from the environment for their development and digestion is the establishment of the utilization of organic entities for contamination clearance (Hamer, 1997; Saval, 1999; Wagner & Amann, 1997; Doble et al., 2004; Gavrilescu, 2005) (Table 15.1).

Phytoremediation

Phytoremediation, also known as plant-based bioremediation, is now utilized to eliminate metallic element from contaminated soil and underground water and other contaminants. Scientists are optimistic that some plants can be used to clean

Table 15.1 Lists a survey of the microorganisms used in environmental restoration

Microorganisms	Type	Shape	Example	Abilities	References
Bacteria	Cocci	Spherical	Streptococcus	Microorganisms that break down hydrocarbons Milk industry waste degradation	Atlas (1981), Leahy and Colwell (1990), Ince (1998), Donkin (1997) and Grady et al. (1999)
	Bacilli	Rods	Bacillus subtilis	Chlorpyrifos-contaminated soil bioremediation Crude oil degradation	Gallert and Winter (1999), Das and Mukherjee (2007) and Lakshmi and Khann (2008)
	Sheathed bacteria	Filamentous	Sphaeratilus	Iron is reduced to ferric hydroxide	Sukla and Panchanadikar (1993), Smith et al. (1994) and Sasaki et al. (2001)
	Budding bacteria	Filaments or hyphae	Hyphomicrobium	One-carbon compounds are necessary for soil and aquatic habitats to flourish.	Trejo and Quintero (1999), Gallert and Winter (2005), Burton et al. (2002) and Duncan and Horan (2003)
	Bdellovibrio	Flagellated	B. bacterivorous	Independently cultivate on intricate biological media	Bitton (2005)
Archea	Crenarchaeotes Euryarcheotic Korarchaeota	Extremophiles	Thermophiles Hyperthermophiles Psychrophiles Acidophiles Alkaliphiles	Prokaryotic cells Use CO_2 as a carbon source and organic molecules	Burton et al. (2002), Bitton (2005) and Doble and Kumar (2005)
Eukaryotes	Fungi	Long filaments	Phycomycetes	Appear on the surface of aquatic plants and animals, as well as certain terrestrial ones	Hamer (1997) and Burton et al. (2002)
	Algae	Floating unicellular microorganism	Phytoplankton	In aquatic habitats, act as primary producers. Develop in mineral medium with vitamin additions, perform oxygenic photosynthesis,	Chavan and Mukherji (2010)
Viruses	Belongs neither to prokaryotes nor to eukaryotes		Viruses	These remain telltale signs of adulteration. Break down host cells	Duncan and Horan (2003)

Table 15.2 Types of phytoremediation

Method	Mechanism	Surface medium
Phytoextraction	Metal concentration & absorption by straight uptake into plant tissue, followed by plant exclusion	Soils
Phytotransformation	Natural compound assimilation and breakdown in plants	Surface water, and underground water
Phytostabilization	Metal precipitates as an outcome of root exudations, diminishing its accessibility.	Soils and groundwater
Phytodegradation	Advances bacterial decay in the rhizosphere	Groundwater and soils in the rhizosphere
Rizofiltration	Metal absorption by plant roots	Surface water
Phytovolatilization	Selenium, mercury, and volatile organic compounds are evapotranspiration by plants.	Soils and groundwater
Vegetative cap	Plants evapotranspiration rainwater to stop toxins from disposal sites from seeping into the soil.	Soils

industrial waste since they have been proven to absorb hazardous metals (Tripathi et al., 2020).

Maintainable methodologies should be formulated to resolve the perplexing issues of contaminated targets, along with other close normal cycles and firmly watched regular attenuation techniques. Various phytoremediation practices characterized in Table 15.2 (Table 15.3).

Phytoextraction Phytoextraction is the usage of floras to move and gather contaminants in ground biomass by engrossing them from soil or water (Salt et al., 1998; Jacob et al., 2018). The main phytoremediation technique utilized nowadays to eliminate heavy metals and metalloids from defiled soil is phytoextraction (Ali et al., 2013; Sarwar et al., 2010). There are a few steps involved in the phytoextraction of heavy metals: Immobilization of toxic metals in the rhizosphere, (ii) utilization of toxic substances by roots, (iii) excretion of ions containing heavy metals from roots to aerial parts of plants, and (iv) sequestration and separation of metal ions in plant tissues (Ali et al., 2013).

Phyto Stabilization Metal tolerant plant class are employed in Phyto stabilization to restrain heavy metals underground and lower their bioavailability. This prevents the metals from migrating into the environment and lowers the risk of metals getting further into the food chain (Marques et al., 2009). Heavy metal precipitation or a decrease in metal valence in the rhizosphere, absorption and sequestration within root tissues, or adsorption onto root cell walls can all result in phytostabilization (Ginn et al., 2008; Kumpiene et al., 2012; Gerhardt et al., 2009).

Phytodegradation Both terrestrial and aquatic plants absorb, store, and biochemically break down or change organic substances into unharmful byproducts or materials for new products. Plant biomass, also known as byproducts, is material that is

Table 15.3 Comparison between traditional and new biotechnology processes and products transformed by creating biotechnological phases

Product	Traditional processes	New biotechnological process	Benefits
Detergent	Using phosphorus as a brightening and cleansing agent	Enzyme-producing microorganisms or fungi that have undergone genetic engineering Use of biotechnological enzymes as cleaning and brightening agents: Proteins are removed by proteases. Lipases eliminate grease marks. Amylases clear off stains from starch	Removing phosphate contamination from waterways Whiter, more vibrant clothing that washes in water with low temperature Energy conservation
Bread	As a preservative and dough-strengthening agent, potassium bromate, a substance that is thought to cause cancer at some concentrations, was added.	Genetically modified microorganisms that generate baking enzymes adding enzymes from biotechnology to: 1. Improve rising 2. Enhance dough 3. Maintain freshness	1. Extended shelf life 2. Absence of potassium bromate
Vitamin B2	Glucose is the first stage in the production process, and then six chemical processes use risky chemicals and produce hazardous waste. Toxic substances used in chemical syntheses, such as aniline	a microorganism that has been genetically modified to generate vitamin B2 (directed evolution) Hydrogenated fat and carbohydrate are utilized as feedstock in a single phase of the fermentation process. Using a genetically modified strain of the gram-positive bacteria Bacillus subtilis, crude riboflavin may be generated directly from glucose. A single fermentation procedure was used in favour of a 10-step chemical process, lowering the number of harmful chemicals used and the amount of acidity in the wastewater generated.	Chemical-free biological production Since it uses a renewable raw material, it uses fewer chemicals (glucose) Reduced expenditures land-based disposal of hazardous waste by 50%

(continued)

Table 15.3 (continued)

Product	Traditional processes	New biotechnological process	Benefits
Paper bleaching	To produce pulp for paper manufacturing, wood chips are simmered in a harmful chemical solution and afterwards treated with chlorine to bleach.	Enzymes are created by genetically modified microorganisms that bleach wood (rDNA). During pulping, enzymes specifically break down lignin and disintegrate the walls of the wood cells.	1. Reduces the amount of hazardous dioxin in the environment and the usage of chlorine bleach. Chlorine elimination in wastewater of up to 15% 2. A decrease in energy use of up to 40% 3. Economically beneficial because energy and chemical expenses are lower
Antibiotics	Chemical manufacturing of antibiotics using chlorinated solvents as well as potentially harmful substances	a genetically modified bacterium created to provide the essential antibiotic intermediate (rDNA) direct fermentation is used in a one-step biological process to create an antibiotic intermediate.	Decrease in energy use by 65% Overall reductions in costs Minimal damage to the environment
Plastic	Petroleum is utilized as a substrate and is broken down into monomers. Many processes are involved in polymerization, and plastics are produced thereafter from polymers.	Use lignocellulosic biomass, corn wastes, straw, or plant sugars. The procedure uses plant-based carbon to generate the PLA polymer.	PLA polymers degrade naturally. Decrease in petroleum use of up to 80%

further broken down into less toxic substances by microorganisms and other processes. Enzymes that promote growth and senescence, sometimes in succession, are involved in the metabolism or detoxification of plants (Yan et al., 2020). Different plant sections may sequentially use oxidative and reductive enzymes.

Phytovolatilization A phytoremediation technique known as phytovolatilization uses plants to absorb pollutants from the soil, transform those harmful substances into less dangerous volatile forms, and then release those substances into the environment by plant transpiration via the leaves. This method may be used to detoxify organic contaminants as well as certain heavy metals, such as *Se & Hg* (Mahar et al., 2016).

Rizofiltraton substances absorbed, quickly absorbed, or precipitated by roots (rhizofiltration), as well as substances absorbed by fungus, algae, and bacteria (biosorption mostly) electrostatic attraction and the development of complexes to cell walls).

Polysaccharides, uronic acids, and particularly sulfated polysaccharides—biopolymers that bind heavy metals are abundant in marine algae. Metals may make up about 10–60% of a plant's dry weight.

Environmental Biotechnology for Contamination Dodging and Cleaner Production

It is believed that biotechnology is the force behind comprehensive environmental protection. Using a variety of approaches, such as:

(a) Utilizing more productive raw materials
(b) As an option in contrast to lessening contamination, which battles for the finish of the cycles and handles contamination after it has been shaped
(c) Mitigation attempts to halt pollution at its source.
(d) Exchanging potentially toxic materials for less damaging ones
(e) Removing harmful chemicals from the manufacturing process

Preventing hazardous pollutants before and throughout industrial production processes is the first step in today's environmental protection strategy.

Doble et al. (2004) provided the following description of an ideal process:

Advantages and Limitations of Phytoremediation

Compared to other corrective measures, phytoremediation has various benefits. Traditional ex-situ phytoremediation techniques, which are more costly than in-situ phytoremediation, involve digging up the soil, off-site storage, cleaning the soil, and covering it in place for stability. Protocols for in-situ phytoremediation are simple to establish, and upkeep is inexpensive. If the plants are properly chosen and the proper agronomic techniques are used to limit the Phyto availability of organic contaminants, phytoremediation has a lot of potential as a sustainable, solar energy-driven in situ option to remediate soils and sites moderately contaminated over wide areas.

Also, in situ phytoremediation adds natural substances, supplements, and oxygen to the soil through both microbial and plant metabolic exercises, upgrading the general condition and surface of remediated regions. Plants utilized in phytoremediation will likewise forestall disintegration brought about by wind and water since they cover the ground and support the soil with their foundations. Every location in any region where plants may thrive can be treated with phytoremediation. A further benefit of phytoremediation is that it enjoys high public approval and is a desirable alternative for businesses and regulators. Moreover, suitable species of plants, ecotypes, types, or cultivars can be chosen, customized, and used for brownfield cleanup, residential or industrial wastewater photo treatment, and remediation of contaminated soils, sites, and cultivars.

Challenges in Phytoremediation

The slower pace of remediation is the main limitation of phytoremediation technologies like excavation and ex-situ treatment. Contaminated soil hinders plant survival and development, which reduces the number of toxins that can be broken down. The endurance and development of plants (utilized for phytoremediation) in the ground may likewise be hampered by a few abiotic (temperature, light, nourishment, precipitation, climatic CO_2) and biotic variables.

These either abiotic or biotic variables can limit plant development, which would therefore hurt phytoremediation efforts. For instance, an attempt at phytoremediation for soil and groundwater at the NASA Kennedy Space Center's hydrocarbon burn facility failed due to water stress and competition from plants. The effectiveness of phytoremediation may also be restricted by physical difficulties. For instance, increasing the moisture availability in contaminated sites that have been exposed to organic pollutants may exacerbate the issue after they have been left dry, inhibiting seedling development as well as growth. How much soil is defiled with specific contamination of interest, just like the case generally speaking; the typical profundity came to be established roots is commonly 50 cm, which is one more huge limit of the phytoremediation strategy. It is crucial to excavate this. The current state and future plans for the phytoremediation of organic contaminants are one sort of problem before phytoremediation. Trees with deeper roots can be repaired at greater depths. In terms of remediating soil and ground, dendroremediation of explosives showed considerable potential. In vitro and greenhouse settings, the use of genetically modified organisms for pollutant removal has shown remarkable results; nevertheless, the regulatory concerns raised have limited their assessment in a natural field context. There is a high likelihood that an interesting gene will be transferred from created organisms to native species in the absence of any effective containment methods.

Therefore, it was not possible to consider releasing the altered strains that carried the antibiotic genes into the environment. According to reports for Europe, if the issue of genetic manipulation from designed to native species could be resolved, the issue of poor social acceptability may appear as the primary barrier to the introduction of created creatures.

Biosensors

Building monitoring systems are required to continuously monitor the environment. This necessitates the creation of new technology and appropriate field methodologies. The biosensors seem like viable choices in this situation. Environmental monitoring biosensors are analytical tools that use a biomaterial, chemical element, or a mix of the two as a sensing element (Gieva et al., 2014). Advanced biosensing devices were created as a result of the need to use some quick, picky, sensitive,

accurate, in-the-moment instruments for detecting and screening contaminants. A biosensor is a type of analytical gadget that employs an immobilized biological component to react with an analyte; a transducer then transforms the biological signal that results into a readable signal. Because they have many advantages over traditional procedures in the field of analysis, biosensors are quite important. Biosensors are employed and used in a numerous areas like agriculture, industry, climate monitoring, military, health & pharmaceuticals (Suryan, 2017). Both organic and inorganic contaminations are produced because of concentrated anthropogenic exercises in manufacturing, agriculture, and other areas. Utilizing biosensors to distinguish contaminants including pesticides, possible dangerous components, microbes, cancer-causing agents, and endocrine-upsetting synthetic mixtures requires steady checking of the genuine states of soil and water specimens. Biosensors wind up utilizing the catalyst's ability for biorecognition. As indicated by Sethi (1994), the electronic parts, or transducers, can be extensively partitioned into four gatherings: electrochemical, optical, piezoelectric, and calorimetric (Fig. 15.14). Each of these gatherings depends on deeply grounded innovation and measures a different quality. For instance, electrochemical transducers report on changes in flow when voltage is held consistent or on changes in flow when voltage is held steady, though piezoelectric biosensors evaluate changes in mass (amperometric). To quantify a critical event, the legitimate natural and electronic parts should be coordinated. The species and quantities of microorganisms in the climate can be distinguished by utilizing biosensors. Water, soil, and air are the significant means for natural observing, be that as it may, there are various more objective analytes (Chocarro-Ruiz et al., 2017). Biosensors are comprehensively characterized into three fundamental classifications represented as follows (Fig. 15.15):

Heavy metals do not biodegrade. Lead, zinc, mercury, cadmium, and copper are the heavy metal pollutants in the surroundings that are most frequently reported. Generally speaking, nitrites are utilized to fertilizes the soil and protect plants. However, is not reliable for human health because it may have several harmful effects. As a result, biosensors have been created that use cytochrome C protein as a sensitive element and amperometric measurement to record different nitrite levels. Pesticides are typically found in the air, water, and soil. Various classes of hydrocarbons, aromatic blends, chlorine complexes, and surfactants are usually found in industrial effluent (Gavrilas et al., 2022). Two distinguishing variables are continually reviewed to investigate the concentration of organic materials in marine habitats. First, the biochemical oxygen demand (BOD) index, estimates the magnitude of oxygen that aerobic microbes utilise to decay biodegradable organic materials. The second factor is the chemical oxygen demand (COD), which reckons how much oxygen is being depleted by chemical methodologies in the water (Wacheux, 1998). Some organic pollutants in effulent, like triclosan, serve as a mark for molecular imprinting polymers (MIPs) and can be integrated with surface plasmon resonance (SPR) to develop a steadfast detection strategy. Electrochemical bio-receptors, such as enzymes, antibodies, and whole cells, can also be employed to detect organic pollutants in effluent. The amperometric biosensors are the most effective method in this category. For pesticides and herbicides, government limitations have been set

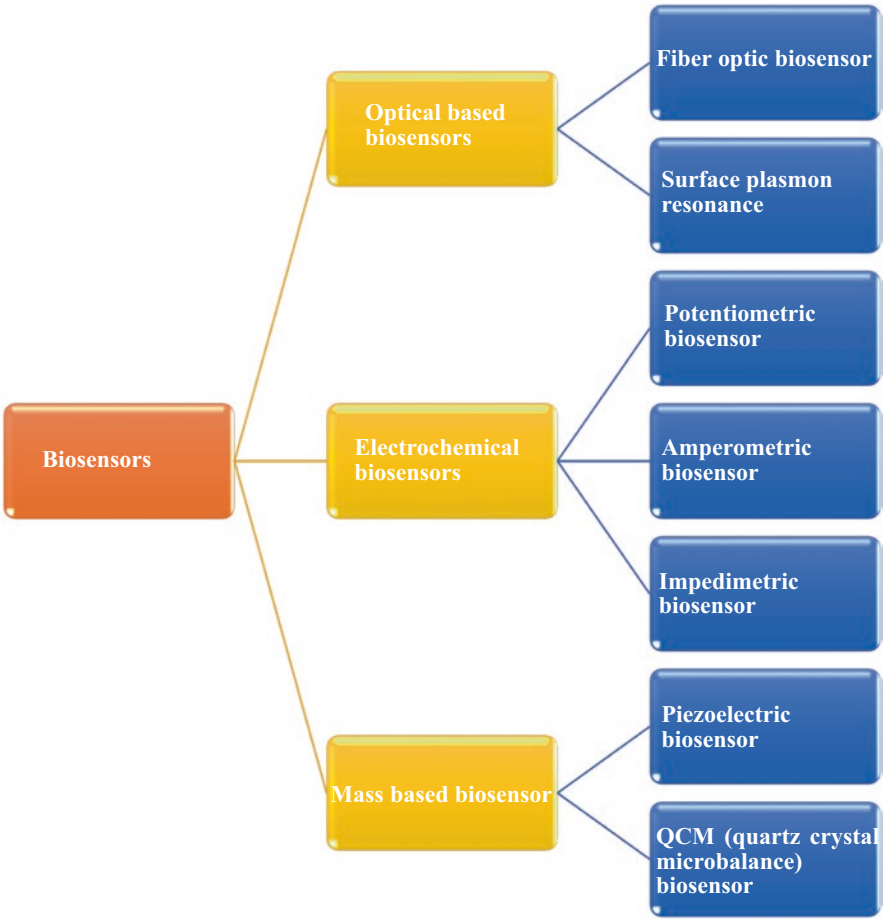
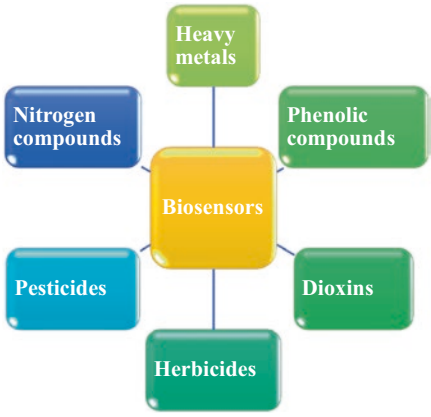


Fig. 15.14 Classification of biosensors

Fig. 15.15 The graphic illustrates multiple categories where biosensors are used for Detection



because of their toxicity to guarantee effective environmental monitoring. Yet, a lot of pesticides harm human health. For instance, wide-spectrum insecticide parathion (O, O-diethyl-O-4-nitrophenyl thiophosphate) is also an extremely hazardous pesticide. Dioxins like polychlorinated biphenyls are generally derived from industrial operations and induce air and water pollution. They require hundreds of years to dissipate. Plants and microbes both create phenolic compounds, but many of these chemicals are poisonous and are thought to be harmful to both humans and the environment. Chlorophenols, which can be found using a chemiluminescence fibre-optic biosensor, are one such example (Gieva et al., 2014).

Biosensors offer considerable potential for the Detection of pathogens and associated toxins in food; yet, they are not frequently used for food microbiological investigation (Vigneshvar et al., 2016). The microbial investigation of food, pathogens includes E. coli, Staphylococcus aureus, Salmonella, and Listeria monocytogenes as well as different microbial toxins like staphylococcal enterotoxins and mycotoxins. A particular biological recognition component and a transducer for signal processing are the basis of the technology (Ejeian et al., 2018). Increasing the output or improving agricultural product preservation is a prevalent goal for the producers.

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Chapter 16
Food Contact Surfaces, Risk of Contamination, and Solution

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Introduction

Given the growing population, the primary goal of the various food industries worldwide is to increase production and ensure food security. However, various impediments stand in the way, and the target is often compromised due to several major reasons, the most serious of which is food contamination. Microbe-contaminated food not only reduces fresh food quantitatively but also has a negative impact on humans upon consumption. Pathogens can enter food at any food processing stage. However, keeping food free of microbes and decontamination through the processing route is the best solution for avoiding the economic, environmental, and harmful effects on human health.

Limiting the survival of microorganisms on contact surfaces is one way to limit their spread. Anything that could come into touch with human food is included in the category of "food contact surfaces," as are any surfaces from where food may be contacted or drained during routine business operations (GMP, 21 CFR 110.3). The most frequent food contact surfaces in various food processing sectors include utensils, knives, workstations, cutting boards, conveyer belts, ice makers, storage containers, gloves, and aprons. Designing of equipment must take care that there is no dead area or poor drainage, but rather sufficient sloping and a proper drainage

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channel. Overall, equipment should be constructed using excellent design principles to facilitate easy cleaning, efficient drainage, and appropriate material compatibility with the food. Material selection for the processing machine should be selected viably, considering material compatibility with the nature of the foodstuff to be processed (Fortin et al., 2021). If equipment material remains in contact with perishable items, it can affect the property of the food. For example, pickled fish can be highly corrosive biomaterial, whereas fresh fish is comparatively weakly corrosive. In the past, wood was considered a good option for food contact materials, but with changing times and needs, new materials have been used as food contact surfaces.

When working with food products that are high in moisture, nutrients, and enzymes, it is essential to clean the surfaces frequently. The meat processing line is one such example, where biofilm formation is quite common; hence, the demand for cleaning at intervals exists. Cleaning is the first step in any procedure involving biological sound materials because previously deposited remains can harbour microbiota and introduce unwanted decontamination into the new batch. After each batch processing, thorough equipment cleaning should be ensured. Nevertheless, other factors, in addition to cleaning inspections, play a role in determining the overall effectiveness of the assurance for the sanitization of food contact surfaces (Owusu-Apenten & Vieira, 2022). Material surfaces should be manufactured with a superior smooth surface so that it can only aid in cleaning and prevent biofilm formation and harbourage niches for microbes, allergen residues, and other contaminants (Faille & Carpentier, 2009; Hasnan et al., 2022). Therefore, every sector of the food industry should adhere to a standard operating procedure that includes the oversight of cleaning tasks, verification checks, and necessary monitoring for visual inspections.

Different global markets have different regulatory requirements for food contact surfaces and materials. Therefore, many nodal agencies aim to improve food contact surface safety by developing standards, validating, and certifying the food equipment that meets the federal requirements for food processing equipment of almost any region. In the United States, National Sanitation Foundation International (NSF International), a not-for-profit-organization, provides 3-A sanitary standards dedicated to maintaining advanced food safety through sanitary equipment design. It also develops uniform, consensus-based national standards or protocols for food processing equipment and packaging that meet almost any region's material requirements. 3-A Symbol/NSF mark on any food equipment confirms that equipment is tested and audited by an independent third party and complies with the stipulations of the FDA Code of Federal Regulations or European Regulations. However, the European Hygienic and Design Group (EHEDG), a consortium of equipment manufacturers, food processing companies, educational institutions, and healthcare officials, has greatly scaled up hygienic engineering in Europe in order to promote hygiene while foods are being produced, processed, and packaged. Before the equipment can display the 3-A Symbol/NSF mark/EHEDG logo, any deficiencies discovered during an inspection must be corrected.

Important Aspects Associated with Food Contact Surfaces Decontamination

Growing concern for safety and imposing strict regulations have led manufacturers to focus more on hygiene maintenance. Substandard designed and/or maintained equipment only adds to the vulnerability of the issue. Henceforth, cross-contamination of food from a contact surface can only be stopped by paying attention to the prerequisites for the workstation including material selection, the right design, and the cleaning of the contact surface before and after use.

Prerequisite for Material Selection and Suitability

Principally, food contact surfaces should comply with regulations directed by the European Union and the Food and Drug Administration (FDA). Surfaces should be non-reactive with both food products and cleaning agents. It should be non-polluting, non-corrosive, non-toxic, non-absorbent, mechanically stable, and easily cleanable. Typically, the working surface must be free from wood and standard glass in open processing areas; however, polymer materials like polycarbonate or reinforced glass (regular glass with a protective layer) are still preferred. The most important consideration when designing any equipment that will be in direct contact with the food is that it does not introduce toxicity to the food. The designer must ensure that no harmful substances enter the food through direct or indirect contact under the intended conditions (temperature, pH, and humidity) (Moerman & Partington, 2016; Moerman, 2017). Worldwide, different federal agencies have established directives that cover material compositions. GMP (Good Manufacturing Practices) for materials and items intended to get into exposure to food throughout Europe are governed by the Food Directive 89/109/EEC, Regulation (E.C.) No. 1935/2004, and Regulation (E.C.) No. 2023/2006. Despite the fact that the member countries of the EU are free to enact their own laws, Regulation (EC) No. 764/2008 of the European Parliament and Council on July 9, 2008 stipulates that every member of the EU must concur on the principle of Cooperative Identification (Lewan & Partington, 2014). While Regulation (E.C.) No. 1935/2004 is for particular obligations on tracing and approval procedures for fresh substances, Framework Regulation (E.C.) No. 1935/2004 on materials and items destined for consumption in nearby nourishment gives general guidelines for governing any kind of food contact matter. Additional legislation involves those governing plastics and items that come into touch with food (Regulation E.U. N° 1183/2012, Regulation E.U. N° 10/2011, and Directive 2002/72/E.C.), contact between recycled plastics and food (Regulation (E.U.) No. 282/2008), elastomers and rubbers in interaction with food (Resolution A.P. (2004) 4 and Directive 93/11/EEC), certain epoxy resins in food contact (Regulation (E.C.) No 1895/2005, and Directives 2004/13/E.C. and 2002/16/E.C.), monomers of vinyl chloride in food contact plastics (Directive 78/142/EEC), and

ceramic components in food contact (Directives 2005/31/E.C. and 84/500/EEC). Chemicals must be properly screened for their effects on human health and the environment under REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals), a law enacted by the European Union. In the U.S., FDA is the nodal agency that directs guidance communicated in the FDA Code of Federal Regulations (CFR), Title 21, parts 174–190 (Hennessey et al., 2011; Skjöldebrand, 2013; Moerman & Partington, 2014; Meghwal et al., 2017).

Food Contact Material

Food contact materials selection and fabrication must adhere to strict guidelines. When selecting a material, it is important to consider the working conditions, including temperature, pH, moisture, pressure, steam, porosity, corrosiveness, and non-tainting. Material suppliers are responsible for making sure that the materials they supply meet the federal requirements for evidence provision. Although there are many materials that can be used for machinery, the choice of material is crucial to the overall effectiveness of the equipment.

Metals are the most important group of materials utilized in the manufacture of machinery and equipment. The metal selection depends on the stress value of the metal, apart from its corrosion resistance, workability, weldability, and cost. Metal construction equipment for wet-cleaned processing stations is logically preferred; however, alloys can also be employed, contingent on the intended working environment (Moerman & Partington, 2014). Surfaces that are bound to come into contact with food should be made of stainless steel that meets the American Iron and Steel Institute's 303, 304, or 316 Series standards or the corresponding Alloy Cast Institute kinds. The chemical compositions of these stainless steel are specified by the American Society for Testing and Materials specifications (3-A SSI 606-05, 2002). Austenite-based 18%Cr/10%Ni AISI 304 stainless steel is recommended for a variety of applications, especially in environments with lower halide ions levels. In such cases, pitting, corrosion, and stress corrosion cracking are quite prevalent. Where the environment contains elevated chloride levels (0.015–0.05%) at optimal working temperatures (<60 °C), the molybdenum comprised 17%Cr/12%Ni/2.5%Mo AISI 316 is recommended. This substance also offers improved resistivity to corrosion, which makes it perfect for drives, rotors, pump castings, and closures. However, because it is so simple to weld, its low-carbon variant, AISI 316L, is suggested for pipelines and vessels. In the case of more complex options, such as cutting blades, AISI 420 or AISI 440C may be required. For harsh working conditions, super-austenitic stainless steels, such as 254SMO offer improved chromium, nickel, molybdenum, copper, and nitrogen contents. This improves corrosion resistance. Apart from that, Incoloy 825, with integrated chromium and nickel content, or even titanium, can be appropriately selected while aiming for corrosion resistance, hardness, pliability, machinability, welding ease, and cost as well. Overall, AISI is commonly used worldwide to manufacture food-grade processing units (Lewan &

Partington, 2014). When it comes to aluminum, it is not recommended due to its insufficient corrosion resistance. However, aluminum along with its alloy can be used as contact surfaces for dry material workstations. Similarly, carbon steel can substantially be considered for dry processing chambers and dry-cleaning operations (Moerman, 2011; Moerman & Partington, 2016).

Plastics are wonderful materials that offer certain advantages over metals, including lower costs, less weight, and better chemical resistivity. However, only a few types of plastic are permitted for use in contact with food, thus when selecting a material, one must ensure that it complies with all applicable laws. Some plastics are porous; hence, there is a risk of food residues and cleaning solutions leaching into the porous materials and then back into the food. Plastics often degrade over time, and additional stress, strain, and temperature, from working conditions and cleaning solutions, hasten the degradation process. Polypropylene, polyvinylchloride, polycarbonate, and high-density polyethylene are typically as for food contact purposes in view of unambiguous cleaning. Moreover, fibre-reinforced and glass-reinforced plastics are increasingly adopted for embodying conveyor belts and for storage of raw materials (Baker, 2013; Djekic et al., 2018). Polytetrafluoroethylene, for example, is said to be porous and hard to clean. Because of this, it is rarely recommended for aseptic packaging equipment. The basic strength and other properties of polytetrafluoroethylene can be improved by the addition of composites. For example, polytetrafluoroethylene (PTFE) is allegedly porous and cumbersome to clean. Therefore, it is often not recommended for aseptic packaging equipment. Composites can be introduced to ameliorate the basic strength and other characteristics. ASTM standards can be used to check for custom-made changes to the composites.

According to the International Union of Pure and Applied Chemistry (IUPAC) definition, elastomers are genetically polymers that exhibit “rubber-like elasticity”. Elastomers have significantly impacted the field of food contact surfaces, with key applications including sealing gaskets, gloves, conveyor belts, and tubing (Kühne et al., 2021). Elastomers comprise an array of chemically different polymers. Rubber represents a group of materials that are distinguished by their elasticity (resilience). Natural rubber, isobutylene-isoprene rubber, acrylonitrile butadiene rubber, and styrene-butadiene rubber are a few examples (Lewan & Partington, 2014). Again, the selection should be made based on the test results in conformity with ASTM standards.

Besides the ones mentioned above, other types of materials are also commonly used for food contact surfaces. Ceramics are often tagged as non-metallic and inorganic materials formed by the action of heat. Clay is one of the oldest known ceramics since historic times. Ceramics are effectively used as active mechanical seal elements on rotating equipment. Adhesives are used to keep gaskets intact at a particular place. They should particularly follow the equipment guidelines, as they often incite parochial corrosion. Open adhesive areas can attract dust and dirt; therefore, no open spaces are acceptable and bonding must be continual, mechanically sturdy, and temperature resistant.

Role of Design and Construction to Minimize Food Contamination

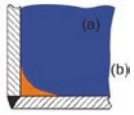
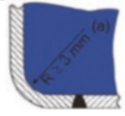
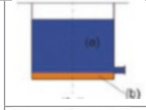
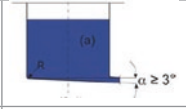
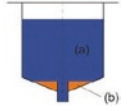
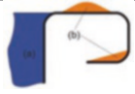
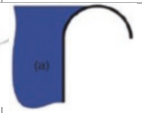
Hygienic design is an easily cleanable design. Specifically, it's not hygienic until cleaned or disinfected, so it's about the design that offers easily accessible cleaning. We find recalls and hazards that sometimes occur in the food industry and directly or indirectly are associated with improper use of equipment. It can be anything; the design of processing, storage, and packaging equipment and its use ultimately impacts. Different aspects that are important to consider while intending to frame equipment are depicted in Fig. 16.1.

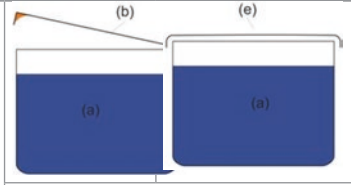
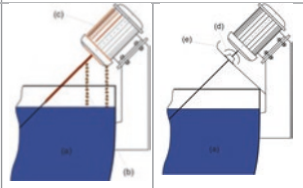
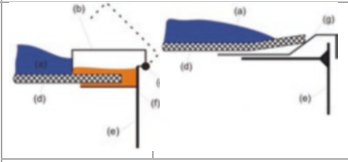
Automation systems would handle process-control activities that ensure food safety and quality. By reliably enabling more complicated activities, they would also simplify the physical design and inventory of physical equipment, reducing construction, cleaning, preventive maintenance, inventory, and dependability risks. In order to guarantee safe food, the processing and handling equipment for food items must be planned, manufactured, constructed, and installed. As a result, surfaces are protected from everyday contact with caustic food ingredients (Faillea et al., 2018). Individual equipment needs, such as joints, drainability, top rims, covers, positioning of auxiliary equipment, sides of conveyer belts and cladding, structure, and insulation, must be considered to lower the risk of food contamination. Hygiene hazards from features like protrusions, recesses, edges, and fissures may be reduced by using permanent joints instead of demountable ones. Welding is the preferred method for permanent couplings between metal parts. However, several other typical flaws may occur hitched welds, including misconfiguration, splitting, porousness, and inclusions, which could turn out out be provenance of microbial loads. Table 16.1 illustrates that welding should not be performed near equipment with sharp corners. Therefore, it is preferable to weld seams in the plane area. When the radius of a corner is limited to 3 mm or less, its cleaning capacity should be evaluated (EHEDG Doc 8, 2018; Moerman, 2011; Moerman & Lorenzen, 2017; Marriott et al., 2018; Schmidt & Piotter, 2020). Another factor that influences food safety is drainability. Equipment used to store food, such as tanks, vessels, troughs, reservoirs, hoppers, bins, and chutes, must be completely self-draining, as shown in the table. Sharp edges must be avoided for proper drainage and cleaning. The radius must be properly determined. Horizontal surfaces must have a slope of at least 3 degrees. The top rims of product-containment equipment should not have ledges where the product may collect and become difficult to clean, especially in open tanks, chutes, and boxes (EHEDG Doc 13, 2004; ISO, 2002). Open-top rims must be rounded and sloping for drainage, as shown in Table 16.1.

Covers are also placed on tanks, transport system edges, and inspection tables to keep items clean while being processed or stored. If they are not completely removable for cleaning, they must be slanted for drainage. If hinged covers are utilized, the hinges must be made to be easily cleaned and to prevent the accumulation of item, dirt, and foreign items like bugs. Continuous-style hinges are not permitted as shown in Table 16.1. It is necessary to properly weld or seal any pipes and devices

Fig. 16.1 Key points to consider while designing or selecting a hygienic equipment design

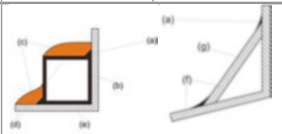
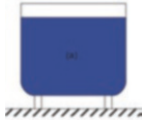
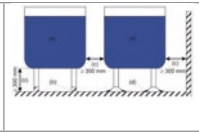
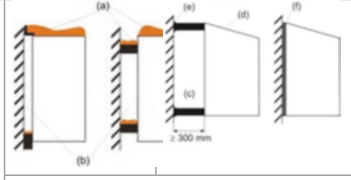
Table 16.1 Exemplification of the importance of design and construction considerations for open equipment to minimize food contamination

Component of food-containing equipment	Don'ts	Do's	Potential outcomes	References
Joints			Sharp internal angles should be avoided as mass pile-up can instigate there, contaminating the batches.	EHEDG Document No. 13 (2004). Hygienic design of equipment for open processing
	(a) Product area; (b) Sharp internal angle			
Drainability			Discharge openings must be fully self-drainable. Discharge outlets above the lowest level of equipment will prevent self-draining and cause residue silting.	
				
	(a) Product area; (b) Residue accumulation			
Top rims			Open tanks and chutes must avoid ledges where the product can become lodged and become difficult to clean. Open-top rim designs must be rounded and sloped for drainage.	
				
	(a) Product area; (b) Possible residue accretion zones			

Covers	 (a) Product area; (b) Pivoted area; (c) Hinge; (d) Dead area; (e) Not fixed	Continuous-style hinges shall not be used. Detachable, unfixed covers are preferable.	
Arrangement of ancillary equipment	 (a) Product area; (b) Contamination – condensate, lubricants; (c) Motor with fins – dead areas; (d) Thrower ring; (e) Self-draining protection sheet with "upstand"	The mounted motor drive above the product should preferably be placed beside the equipment rather than above it.	
Sides of conveyor belts	 (a) Product area; (b) Pivoted cover; (c) Hinge; (d) Belt; (e) Frame; (f) Dead area; (g) Detachable cover	Non-removable bearing surfaces for belts and covers, as well as hinges of pivoted covers, are difficult to clean.	

(continued)

Table 16.1 (continued)

Component of food-containing equipment	Don'ts	Do's	Potential outcomes	References
Cladding, framework, and insulation			Possible soil retention in horizontal ledges, projections, and frames. Sloped surfaces avoid the accumulation of soils and assist drainage. A minimum slope of 30° is required to avoid dust accumulation and facilitate inspection. Cladding must be installed to maintain a minimum clearance of 30 cm between adjacent surfaces.	
	(a) Welding; (b) Frame; (c) Residues of soil; (d) Horizontal parts; (e) Cladding; (f) Slope; (g) Reinforcement			
Installation of equipment fixed to floors			Complicated cleaning underneath the equipment with a small clearance to the floor. Proper fixation of feet to rounded pedestals is ideal or sealed to the floor with sufficient clearance characterizes hygienic design.	
	(a) Product area; (b) Rounded pedestal; (c) Clearance; (d) Sealed to the floor			
Installation of equipment fixed to walls			Horizontal surfaces or ledges retain soil and small clearances impede cleaning between walls and equipment. Horizontal equipment supports are ideal with underneath clearance; direct fixation of equipment to the wall is better if sealing materials are used.	
	(a) Residues of soil; (b) Small clearance; (c) Clearance; (d) Slope; (e) Radius; (f) Sealing			

Adapted from DOC 13: Hygienic design of open equipment for processing of food

that are attached to or going through covers (Moerman & Kastelein, 2014; Gurnari & Gurnari, 2015). When a motor drive is installed above a product, it is preferable to install it next to the equipment rather than above the product. Drip trays and discharge rings on the drive shaft are necessary for other appliances to reduce the possibility of lubricating and dust from the electric motor or gearbox seeping onto the product and contaminating the table. The bottom of the throwing ring must be accessible for inspection. Conveyor belts may meet food when utilized for product inspection or conveyance.

Bearing surfaces that are detachable and simple to clean provides support to the belt's edges (Kold & Silverman, 2016; Rashid et al., 2023). Equipment cladding needs to be uniform, persistent, and crack-free in order to be easily cleaned. Ledges, projections, and crevices should be avoided since they pose a risk of soil retention. If feasible, tilt horizontal shelves and extensions (Table 16.1). Slopes of at least 30 degrees are recommended to discourage dust collection and make routine checks less laborious. A minimum of 30 centimeters must be left between the cladding and any adjoining walls or ceilings. If equipment support structures are connected to the floor or walls, the equipment must be adequately sealed against the mounting surface, or a minimum clearance for cleaning and inspection must be used. After cleaning, it's crucial to keep spaces, fractures, and cracks free of any places where insects or bacteria could hide or thrive. The gap depends on the equipment's bottom size, which should be 300 mm (EHEDG Doc 8, 2018; Lelieveld et al., 2014).

Traditional and Emerging Trends in Food Contact Surface Decontamination

Different factors in food processing realm may favour the formation of biofilms. Moisture and nutrients act as a nucleus to attract microbes, which not only live on them but also present a significant risk of food contamination during the processing line, which resultantly only adds to the transmission of foodborne pathogens and health implications. In reference to this, it becomes critically important to implement procedures to prevent, minimize or eliminate the cause. Conventional procedures do exist, but have few limitations that limit their use. Henceforth, associated individuals and food industrialists are constantly looking for suitable alternatives that can help with these issues while wasting as few resources as possible. An extensive summary of the various trends in the development of technology for the cleaning of food contact surfaces is shown in Table 16.2 and discussed hereunder.

Heat treatment is among the most common and traditional methods for treating food contact surfaces. Heat-based methods are still widely used in industry today. Hot water and steam have been conventionally used to eliminate formed biofilms from surfaces. To clean surfaces, a spurt of hot water is channelized to treat the processing surfaces, specifically, with water temperatures optimized to target a particular lot of microbiotas. A high-pressurized steam is splashed on surfaces,

Table 16.2 Recent approaches on technological advancements for decontaminating food contact surfaces

Approach	Treatment	Surface	Treatment specifications	Results	References
Heat	Hot water	Stainless steel	Hot deionized water 71 °C for 30 s	All biofilms were very sensitive to hot water treatment, which reduced the *S. Enteritidis* cell populations by 4.30–7.08 log CFU/cm^2	Yang et al. (2017)
	Superheated steam (SHS) and saturated steam (S.S.)	Stainless steel (type 304 with No. 4 finish (STS No. 4), stainless steel (type 304 with 2B finish (STS 2B), high-density polyethylene (HDPE), and polypropylene (P.P.)	The coupons were exposed to SHS on both sides for 2, 4, 7, 10, 15, or 20 s. The distance between the coupons and the steam generator nozzle: 7 cm Saturated steam (S.S.) treatments were performed at 100 °C, while SHS treatments were performed at 125 or 150 °C.	Amongst all coupon types, SHS was more effective than S.S. in inactivating the *S. aureus* biofilms. *S. aureus* biofilms on HDPE and P.P. coupons were reduced by 4.00 and 5.22 log CFU per coupon, respectively, after S.S. treatment (100 °C) for 20 s. S.S. treatment for 20 s reduced the amount of *S. aureus* biofilm on STS No. 4 and STS 2B coupons to below the detection limit. SHS treatment (150 °C), S. aureus biofilms on HDPE and P.P. needed 15 s to be inactivated to below the detection limit, while only 10 s for steel coupons.	Kim et al. (2019)
	Saturated steam	Stainless steel (S.S.) (AISI 316, No. 4), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polyethylene terephthalate (PET)	A stainless-steel chamber (34 cm × 57 cm × 29 cm) with three steam distribution pipes and 25 steam nozzles was used for the experiment.	Steam exposure for 30–180 s at 100 °C set off a 4.0–6.4 log10 CFU/coupon reduction of *L. innocua* biofilm on S.S., and 3.0–4.8, 2.8–4.2, 2.7–4.5 and 2.6–3.3 log10 reductions on PET, LDPE, PVC, and rubber surfaces, respectively.	Hua et al. (2021)

Chemical	Chlorine gas	Teflon, silicon, rubber, polyvinyl chloride (PVC), type 304 stainless steel (S.S.) with 2B or No.4 finish, and glass	ClO_2 gas was prepared using a ClO_2 gas generating system and was introduced into the polyvinyl chloride treatment chamber. Inoculated samples were placed in the treatment chamber and covered with a plastic lid. Gas concentration.: 20 ppmv ClO_2 Treatment time: 5, 10, and 15 min Treatment temp: 22 ± 2 °C. R.H. of the treatment chamber: 90%	The degree of log reduction of the three pathogens increased in the following order – silicon, Teflon, rubber, S.S. 2B, PVC, and S.S. No.4. A significantly higher ($p < 0.05$) inactivation of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes,* was achieved on glass with more than 5.91 to 6.81 log reduction (detection limits <0.48 log CFU/cm^2). As treatment time increased, different levels of inactivation of the three pathogens were observed among the samples. Contact angles of food contact surfaces were highly and negatively correlated with the log reduction of all three pathogens. There were generally weaker correlations between the roughness values of sample surfaces and microbial reduction compared to those between hydrophobicity and microbial reduction	Park and Kang (2017)
	Alcohol-based sanitizer (70% v/v ethanol solution) and the chlorine-based sanitizer (200-ppm sodium hypochlorite solution)	Polypropylene (P.P.), polyethylene (P.E.), stainless steel (SUS) and glass (G.L.)	Surface coupons were immersed in 100 ml of chemical sanitizers. Immersion time: 3, 5, or 10 min Temperature: 25 °C.	Sanitization more efficiently lowered *S. aureus* counts on SUS and G.L. than on P.P. and P.E. Sanitization efficacy of ethanol was better than that of chlorine. Surfaces with scratches and biofilms were the most resistant to sanitization methods. Ethanol emerged effective bactericidal agent, regardless of the material and roughness	Kim et al. (2017)

(continued)

Table 16.2 (continued)

Approach	Treatment	Surface	Treatment specifications	Results	References
	Slightly acidic electrolyzed water (SAEW)	Stainless steel and glass	SAEW was generated by the electrolysis of sodium chloride and hydrochloric acid using a flow-type electrolysis apparatus equipped with a non-membrane electrolytic cell. SAEW was diluted to 10-, 30- and 50-fold in the sterilized water used in this study	The results showed that SAEW (pH 5.09 and available chlorine concentration (ACC) of 60.33 mg/L) could kill *L. monocytogenes* on food-contact surfaces completely in 30 s, a disinfection efficacy equal to that of NaClO solutions (pH 9.23 and ACC of 253.53 mg/L). The results showed that long exposure time and high ACC contributed to the enhancement of the disinfection efficacy of SAEW on *L. monocytogenes* on food-contact surfaces.	Hao et al. (2022)
	Slightly acid electrolyzed water (SAEW, pH = 5.0)	Stainless steel	Saturated chloride solution (25 g/L) and tap water were simultaneously pumped into the generator (18–20 °C). The amperage was fixed at 20 A. ACC: 50–200 mg/L pH = 5.93 Oxidation-reduction potential (ORP) = 948 mV Exposure time: 0–6 min	SAEW yielded higher reductions of *L. monocytogenes*, i.e., 2.30 ± 0.16 to 5.64 ± 0.11 log cfu/cm^2, in comparison with neutral electrolyzed water (NEW, pH = 7.0) (1.55 ± 0.11 to 5.22 ± 0.12 log cfu/cm^2), attributable to the synergistic bactericidal effect between the acidic pH, higher oxidation-reduction potential and the effective form of chlorine.	Possas et al. (2021)

Physical U.V.	Ultra-high irradiance (UHI) blue light	Stainless steel, glass, polypropylene, polyethylene	Light electroluminescent diode (LED)-based device was designed to generate irradiation at an ultra-high-power density (901.1 mW/cm^2).	Short-time treatments (below 10 min) at 405 nm induced a ~4.5 log reduction rate of the cultivable yeast population. The inactivation rate was positively correlated to the overall energy received by the sample and, at a similar energy, to the power density dispatched by the lamp. Within 5 min of treatment, *S. cerevisiae* disinfection was achieved for all tested surfaces. The disinfection of stainless steel was particularly effective, with a complete inactivation of the yeast after 2 min of treatment.	Lang et al. (2022)
	UV-C LED	Stainless steel (S.S.) and high-density polyethylene (H.D.)	Wavelength: 250–280 nm Power: 20 mW The lamp was operated under forward bias at a maximum 400 mA current, corresponding to 100% irradiance. The average irradiance: 2 mW/cm^2 or 4 mW/cm^2	*Salmonella* on S.S. was reduced by 1.97 and 3.48 Log CFU/cm^2 after 60 s of treatment with 50% and 100% irradiance, respectively. H.D. showed a lower decrease of *Salmonella*, but still statistically significant ($p \leq 0.05$), with 1.25 and 1.77 Log CFU/cm^2 destruction for 50 and 100% irradiance after 60 s, respectively. Longer exposure times of H.D. to UV-C yielded up to 99.999% (5.0 Log CFU/cm^2) reduction of *Salmonella* with both irradiance levels	Calle et al. (2021)
	UV-C	Polyethylene (P.E.) and stainless steel (S.S.)	Custom-made U.V. unit 95 W low-pressure mercury lamps of 50 cm length housed in an enclosed stainless-steel cabinet with internal dimensions of 790 × 390 × 345 mm. Treatment distance: 6, 16, 26 cm	When S.S. was treated with UV-C, the maximum reduction of *P. fluorescens* achieved was 2 log cycles, even at the highest dosage.	Pedrós-Garrido et al. (2018)

(continued)

Table 16.2 (continued)

Approach	Treatment	Surface	Treatment specifications	Results	References
Sound waves	Ultrasound—steam treatment	Plastic (polystyrene plates, Ø = 52 mm) and steel (Ø = 20 mm)	Ultrasound range: 20–40 kHz Nozzle delivery: 25 kg/steam per hour at 2.7 Bar(g) pressure Temperature: 85, 90 or 95 °C Treatment times: Murine norovirus (MNV) (0.8–2.0 s on plastic; 0.8–5.0 s on steel) and for hepatitis A virus (HAV) (0.8–5.0 son plastic and steel both)	For MNV on plastic and steel surfaces at temperatures 85, 90 or 95 °C, a mean genome copies (G.C.) reduction of log 0.4, 0.2 or 0.3 and 0.4, 0.4 or 0.5, respectively, were observed. For HAV on plastic and steel surfaces the mean log reduction of G.C. observed were 0.8, 0.7 or 1.5, and 0.4, 0.4 or 0.6 at temperatures of 85, 90 or 95 °C, respectively.	Rajiuddin et al. (2020)
	Ultrasound-assisted sodium hypochlorite (NaOCl) treatment	Stainless steel	Ultrasound treatment times i.e., 5, 20, 40, 60, 80, and 100 min, with NaOCl (50, 100, 150, and 200 ppm)	100 min ultrasound treatment solely reduced *L. monocytogenes* of 1.09 on stainless steel, depending on the treatment time (5–100 min). Population reduction ranged 1.48–3.79 CFU/mL, depending on the NaOCl concentration (50–200 ppm)	Lee et al. (2014)
	Ultrasound	Polyurethane conveyer belts	Ultrasound frequency: 37 kHz, 200 W Treatment time: 30 min At room temperature	Ultrasound effectively controlled the overall biomass of the biofilm inoculated from cells and spores from the surface.	Fink et al. (2017)

Plasma	Cold atmospheric pressure (CAP) plasma	Stainless steel and polyvinyl chloride (PVC)	Stainless steel (S.S.) type 304 of 0.18 mm in thickness and black polyvinyl chloride (PVC) of 3.4 mm in thickness Treatment time: 120 s Treatment distance: 3 cm	A 120 s treatment time with a 3 cm treatment distance from the surface reduced both adherent cells (initial 5.6 ± 0.2 log CFU/coupon) and 24 h biofilms (initial 5.8 ± 0.4 log CFU/coupon) on stainless steel (S.S.) by >4.6 log CFU. While the same treatment reduced adherent cells (initial 5.7 ± 0.5 log CFU/coupon) and 24 h biofilms (initial 6.9 ± 0.5 log CFU/coupon) on polyvinyl chloride (PVC) by 3.8 ± 0.9 and 3.5 ± 0.5 log CFU, respectively. Mature biofilms (72 h grown) were more resistant than 24 h grown biofilms. This waterless technique induced no changes in S.S. and PVC in terms of chemical properties and visual topography.	Wang et al. (2023)
	Cold atmospheric pressure (CAP) plasma	Stainless steel, commercial poly[ether]-thermoplastic poly[urethane] (PE-TPU) conveyor belts	The plasma system was evaluated against two common food-borne pathogens (*Salmonella* Typhimurium, *Listeria monocytogenes*) on stainless steel surfaces and against *S* Typhimurium on PE-TPU conveyor belts under simulated conditions of a food-processing facility.	A significant level of microbial inactivation was achieved, up to 3.03 ± 0.18 and 2.77 ± 0.71 log CFU/mL reductions of *L. monocytogenes* and *S.* Typhimurium, respectively, within 10 s total treatment on stainless steel surfaces, and a 2.56 ± 0.37 log CFU/mL reduction of *S.* Typhimurium within 4 s total treatment on the PE-TPU material, according to a procedure based on the well-established EN 13697:2015 industrial protocol.	Katsigiannis et al. (2022a, b)

(continued)

Table 16.2 (continued)

Approach	Treatment	Surface	Treatment specifications	Results	References
	High voltage atmospheric cold plasma (HVACP)	Stainless steel, PVC, and silicone	Power supply output: 60 W Dielectric barriers: 4 pieces of plastic Electrodes: Two 15 cm diameter aluminium electrodes Coupons containing *C. sakazakii* were placed in plastic dishes inside a plastic bag (25.5 × 35.5 cm) flushed with a modified atmosphere (10% air, 90% helium) Treatment time: 0, 30, 60, and 90 s.	90 s treatment effectively reduced *C. sakazakii* by ~3 log CFU/coupon compared to untreated coupons.	Phan et al. (2023)
	Gliding arc discharge (GAD) plasma	Silicone (Si), stainless steel (S.S.), polyethylene terephthalate (PET)	GAD plasma was applied using nitrogen gas at flow rates of 0.5 and 1 m^3/h for different time intervals (5–10 min). All 3 surfaces were artificially contaminated with 8.15 ± 0.28 log cfu/mL of *E. coli* and 6.18 ± 0.21 log cfu/mL of *S. epidermidis*.	Significant reductions of 3.76 ± 0.28, 3.19 ± 0.31, and 2.95 ± 0.94 log cfu/mL in *S. epidermidis*, and 2.72 ± 0.82, 4.43 ± 0.14, and 3.18 ± 0.96 log cfu/mL in *E. coli* on S.S., Si, and PET surfaces, respectively, were achieved after 5 min of plasma treatment by using nitrogen as the plasma forming gas ($p < 0.05$).	Dasan et al. (2017)

	Cold oxygen plasma	Stainless steel	Distance b/w electrode and the sample: 10 cm Ozone production was monitored at a distance of 9.5 cm Ozone generated: 3.0 ppm Emitted U.V. light was measured by a photo-radiometer, with doses of 1210–1250 mW/cm^2 at a distance of 9.5 cm. The maximum temperature reached: 32.5 °C. Exposure time to COP: 0, 10, 30, 60, 90, 120, 180, 240, and 300 s	Decrease in the MNV-1 (a human norovirus [NoV] surrogate) and HAV (hepatitis A virus) titers resulting from 10 to 300 s of cold oxygen plasma were 0.27–3.89 and 0.77–2.02 log PFU/ml, respectively.	Park and Ha (2018)

(continued)

Table 16.2 (continued)

Approach	Treatment	Surface	Treatment specifications	Results	References
	Atmospheric pressure plasma jet	Stainless steel	Input power: 650 W	The maximum *Salmonella enterica* population reduction calculated before the inactivation tail ranged from 3.32 (316 HL) to 4.97 log CFU/in^2 (304 MR). Plasma treatment of metal surfaces resulted in an abrupt increase in surface temperature, reaching up to 180 °C within 15 s of treatment, and led to log-linear inactivation in all surfaces treated with atmospheric plasma jet.	Gabriel et al. (2018)
	Cold nitrogen plasma (CNP)	Stainless steel	CNP dose: 200, 300, 400, 500, and 600 W Exposure time: 0.5, 1, 1.5, 2, and 2.5 min.	Upon 400 W treatment for 1 min with CNP, the number of *S. aureus* biofilm was reduced by 2.02 logs. Similarly, the population of *S. aureus* biofilm on a 96-well plate was reduced from 7.30 logs to 5.61 logs. An evident decline of established *S. aureus* biofilms metabolism was seen after exposure to CNP at 400 W for more than 1 min ($P < 0.05$). The population of S. aureus biofilm was reduced by 2.03 logs after 1-min treatment. Extending the treatment time to 2.5 min, the amount of S. aureus was dropped by 3.02 logs. Correspondingly, the population value was reduced from 7.23 logs to 5.33 logs.	Cui et al. (2016)

Biological	*Cinnamomum cassia* and *Salvia officinalis* E.O. based microemulsions	Stainless steel type 304	*C. cassia* and *S. officinalis* E.O.s were selected to formulate microemulsions at relative MBEC concentration (2.5% and 5%, respectively) The two formulations were stabilized with Tween 20 as surfactant.	In reference to contact times utilized, action of E.O. microemulsions was more effective after 90 min (P b 0.05) against *S. aureus* ATCC 43387 24 h old biofilms and desiccated ones. In fact, after 90 min of contact, logarithmic reductions N3 were obtained with all the E.O. microemulsions against *S. aureus* ATCC 43387 2 biofilms in all the applied culture media, with the only exception of *C. cassia* microemulsion toward desiccated biofilms. 90 min treatment with all the formulated E.O. microemulsions ably removed up to 68% of the biofilms from stainless steel surface, in case of the microemulsion compris3ed with *C. cassia* and S. *officinalis*, but none removed biofilm completely.	Campana et al. (2017)
	Thymol, carvacrol and thymol/ carvacrol liposomes (TCL)	Stainless steel AISI 304	Sanitizing solution: 0.106 g of each compound (Carvacrol and thymol) were diluted in 10 ml of 20% (v/v) ethanol solution. Concentration: 0.5, 1.0, and 2.0 MIC Contact time: 1 and 10 min	Adhered *S. aureus* (± 6.1 log CFU/cm^2) were inhibited after 1-min and 10-min treatments using thymol or carvacrol at minimal inhibitory concentration (MIC) and 2.0 MIC. Reductions of 1.47–1.76 log CFU/cm 2 and 1.87–2.04 log CFU/cm 2 were obtained using 0.5 MIC of thymol and carvacrol, respectively. A 10-min contact with free (MIC and 2.0 MIC) and encapsulated (MIC) antimicrobials inhibited attached *Salmonella* (± 6.0 log CFU/cm^2); however, after 1-min of contact, 2.0 MIC of thymol and carvacrol were not able to inactivate adhered *Salmonella* MIC of TCL inactivated S. aureus and *Salmonella* after 10 min; however, after 1-min contact, adhered *S. aureus* and *Salmonella* populations were decreased in 1.62 log CFU/cm 2 and 2.01 log CFU/cm^2, respectively.	Engel et al. (2017)
	Red globe grape stem extracts (GSE)	Stainless steel 304 and polypropylene	Concentration: 0, 0.125, 0.25, 0.5 and 1 time the MIC	GSE reduced adhesion of *Listeria* to stainless steel (0.77–2.22 log CFU cm^{-2}) and polypropylene (0.71–2.38 log CFU cm^{-2}) and completely inhibited bacterial motility at 4.5 mg mL^{-1} of GSE.	Vazquez-Armenta et al. (2018)

however, its efficacy is conditional to direct access, as steam cannot reach hidden spots (Skåra & Rosnes, 2016). The use of steam is quite prevalent for surface decontamination as steam is a powerful energy carrier upon condensing on certain surface. However, complications are associated with contact time and temperature monitoring throughout the contact. Another issue is that, probably because of the contact surface design, the deactivation rates of interface-attached cells may be different from those of floating freely cells. Amplified temperature ranges, however, can speed up and improve the reactions, and henceforth combinations of heat and other approaches can be beneficial (Basumatary et al., 2020).

Chemical washing is another method to ensure microbial disinfection of food contact surfaces. It is often carried out with antimicrobial compounds such as chlorine, iodine, hydrogen peroxide, and quaternary ammonium compounds (QACs) approved chemicals for sanitation purposes. Nevertheless, antimicrobial efficacy is primarily determined by three factors: concentration, temperature, and contact duration. Traditional washing has the disadvantage of reacting with the components of the food surface, making them much more harmful (Song et al., 2019). Number of studies have been unveiled on the associated shortcoming of chemical issues. Alcohol-based disinfectants were observed to be effective for welling and hardening of rubber and certain plastic surfaces, but ineffective against some viruses (Chang et al., 2013). There have also been reports of QACs adsorbing onto cotton substrate wiping materials and ultimately limiting the disinfection procedure failure. Peroxygens have been known to cause chemical irritation and, in some cases, also turned up as corrosive for copper, brass, and bronze surfaces, particularly Peracetic acid (PAA) (Jennings et al., 2015). Prolonged chlorine application is harmful to the outer plastic coat of some insertion tubes (Song et al., 2019). Secondly, the potential reach of chemical washes is sometimes insufficient for the hidden points of apparatuses that cannot be dismantled or for unsuitable construction material used in equipment. Also, the incorrect concentration of cleaning and sanitation agents can have a significant impact on the efficiency of the cleanness and disinfection process. In order to effectively reduce harmful germs, the ratio of these chemical compounds must be tuned because a larger concentration could be damaging to human health. Chemical sanitizers are purposefully used in industries and temperature range of 13–49 °C with particular contact length is recommendable for effectiveness against microbial loads (Sharma et al., 2022). The combination of suitable chemicals at a minimum concentration can provide synergistic effects and eliminate the extreme of sole chemical application. One example is electrolyzed oxidizing water (EOW), which is formed by electrolyzing a sodium chloride solution in an electrolysis chamber with an anode and a cathode separated by a membrane. For EOW production, a salt-diluted solution and current are proceeded via chamber, parting the solution into two separate streams, i.e., acid EOW (at the anode) and alkaline EOW (at the cathode). Weak organic acids can also be used for surface decontamination (Meireles et al., 2016). Because a variety of organic acids, such as citric acid, formic acid, lactic acid, and acetic acid, are naturally present in foods, their application is consumer friendly. Many have GRAS status and have been approved by the FDA and European Commission.

Physical approaches are more popular, especially since fast, mild, and residue-free approaches have received more attention. In comparison to chemical approaches, they can be holistically applied across the processing chain and, in some cases, enables the conditioning of various surfaces (Otto et al., 2011).

Non-thermal plasma is currently being studied extensively. The final state of matter is known as plasma, and in the food sector, charged plasma is highly valued for its superior surface cleaning capabilities. Despite having a neutral net charge, plasma is often described as a "quasi-neutral" medium because it is conductive to electricity (due to the presence of free charge carriers). Its ability to produce an antimicrobial effect and its applicability for surface sanitization are both supported by the presence of electrons, atoms, ions, radical substances, and molecules in a fundamental or excited state, including reactive oxygen species, or ROS, and reactive nitrogen species (RNS), as well as electromagnetic energy (U.V. photons and visible light). These are promoted as highly effective against remnants of biofilms, which are again capable of increasing the inflammatory processes in the adjacent tissues (Mravlje et al., 2021). Plasma technology can be divided into generation, thermal, and low-temperature categories. According to many researchers, thermal plasma consists of thermodynamically balanced ions, electrons, and gas molecules at temperatures around 20,000 K. Non-equilibrium plasma is referred to by vernacular names, such as Cold Plasma, Atmospheric Cold Plasma, and Non-thermal Plasma. Cold plasma can be produced by a radiofrequency generator or by atmospheric pressure, while the dielectric barrier discharge, atmospheric plasma jets, gliding arcs, and radiofrequency-based and microwave-based discharges are the most used atmospheric Cold Plasma sources (Ansari et al., 2022; Hernández-Torres et al., 2022). In an investigation by Khan et al. (2016), a dielectric barrier discharge plasma reactor (underwater DBD) was employed for biofilm inactivation on stainless steel caused by three distinct foodborne pathogens. After 90 min of plasma treatment, results included an inactivation of 5.50 log CFU/coupon, 6.88 log CFU/coupon, and 4.20 log CFU/coupon for *Escherichia coli* O157:H7 (ATCC 438), *Cronobacter sakazakii* (ATCC 29004), and *Staphylococcus aureus* (KCCM 40050), respectively. In another study, Aboubakr et al. (2020) reported that using an air DBD against *Salmonella enterica serovar* Heidelberg on stainless steel resulted in only a 2.5 log CFU deduction on dry surfaces in 10 min. In contrast, >6.5 log CFU decrement was attained on wet surfaces in 3 min, with recommendation for apt application after cleaning to eliminate residual water molecules.

Power ultrasound, typically at a frequency of 20 kHz, is yet another technique that can aid in sanitation practices. Pulsating waves move through water because they are much stronger than regular sound waves, creating millions of tiny cavitation bubbles. These are immensely strong wave bubbles that pop and implode. During implosion, matter and energy capitulate. During an ultrasonic procedure, these imploding cavitation bubbles hit an object's surface, generating heat and even more energy. Energy bursts and rebound on the surface, removing things from the object like a high-pressure vacuum. This approach does not require scrubbing, scrapping, or chemicals, and it saves time while being eco-friendly. This ultrasound-based cleaning can be specifically tuned to ensure the sanitation of various

equipment, apparatuses, and parts in the food industry. This method, when combined with heat (thermosonication) or pressure (manosonication), can effectively aid in biofilm elimination. Thermosonication is commercialized for the disinfection of conveyor belts (Musavian et al., 2015; Dallagi et al., 2023). Few studies have been conducted recently to disinfect food contact surfaces to ensure safety from microbes. Webber et al. (2015) used an ultrasound bath treatment (40 kHz frequency and 81 W potency) for 10 min to decontaminate stainless steel AISI 316 coupons (1 cm^2). The authors concluded that ultrasound effectively detached biofilms formed in vitro, highlighting the ease of use and their hydrodynamic properties responsible for destabilizing biofilm structure. In a separate study, Brasil et al. (2017) employed ultrasound (U.S.) with chlorinated water (C.W.) to decontaminate slaughtering knives and compared it to the conventional cleaning method, i.e., manual cleaning with sponges using neutral detergent and washing with chlorinated water (2.05 ± 0.8 mg/l), followed by sterilization (during 20 s at 82.0 °C). The results revealed that the conventional sanitation approach reduced ($p < 0.05$) the counts of mesophiles, *Enterobacteriaceae*, moulds and yeasts, and a similar expression was recorded for U.S. + C.W. (2.05 ± 0.08 mg/l of chlorine, and mode operation normal and sweep for 10 min) and U.S. + C.W. + ND (5 ml/l and mode operation sweep for 5 min) methods. However, increasing the detergent concentration and sonication time (20 ml/l, 15 min) resulted in a significant fall (p 0.05) for the same microbes. Ultraviolet light is another promising dry decontamination technology that is inherently non-thermal in nature. It is a U.S. Food and Drug Administration-approved intervention technique that can be potentially used for effective pathogen decontamination in food contact surfaces and food surfaces. UV gamma irradiation uses UV light technologies to disinfect environmental surfaces. These technologies are portable or stationary units that can disinfect an entire vacant room. Energy emittance from U.V. light ranges between 100 and 400 nm. They travel through waves or particles without causing radioactivity. The classification of ultraviolet light involves UV-A, with wavelengths between 315 and 400 nm, which is linked to human skin tanning; UV-B, with wavelengths between 280 and 315 nm, which is linked with cutaneous burning and cancer of the skin; and UV-C, with wavelengths between 200 and 280 nm, which is known as the germicidal differ due to its efficacy in inactivating bacteria and viruses (Monteiro et al., 2021; Byun et al., 2022). In the food industry, UV-C is used to eliminate microbes on food contact surfaces. UV-C light (200–280 nm) induces alterations in the microbial DNA structure primarily by two different mechanisms: the first is cross-linking genesis between cytosine and thymine, known as direct action, the second is free-radical generation via water radiolysis, known as indirect action. In order to combat microorganisms in the UV-C spectrum, light emittance at 253.7 nm is consistently encouraged because nucleic acids are the primary light absorbers at this particular wavelength (Monteiro et al., 2021; Monteiro et al., 2022).

Several studies on UV application for surface decontamination have been conducted over the last 10 years. Gabriel et al. (2018) tested the potential of UV-C (15 W UV-C light source; lamp to the metal surface distance of 9.8 cm) against stainless steel (304, and 316) with exposure times ranging from 0–180 s. An early

fast, log-linear deactivation stage is followed by a gradual inactivity tail in the observed inactivation behavior. D1 and D2 values, or two decimal reduction times, were identified. The D1 values varied from 2.26 (304 MR) to 4.31 s (304 2B) during the initial quicker log-linear inactivation phase. In comparison to type 304 metals, which had D1 values of 2.26–4.31 s, type 316 metal had slightly shorter values, ranging from 2.54 to 3.51 s. In another investigation, Calle et al. (2021) employed UV-C LED light (250–280 nm wavelength, 20 mW power, 105 degrees viewing angle) at 2 mW/cm^2 (half irradiance) or 4 mW/cm^2 (full irradiance) on stainless steel (S.S.) and high-density polyethylene (H.D.), for surface decontamination targets. After 60 s of being treated with 50% or 100% irradiance, the reduction of salmonella on S.S. was 1.97 or 3.48 Log CFU/cm2, respectively. With 1.25 and 1.77 Log CFU/cm2 eradication after 60 s for 50 and 100% irradiance, respectively, H.D. demonstrated a lesser reduction of Salmonella but was still statistically significant (p 0.05). Salmonella was reduced by up to 99.999% (5.0 Log CFU/cm2) with both irradiance levels when H.D. was exposed to UV-C for longer periods of time. Aside from the associated concerns and issues with the technological application, its performance on rough surfaces is affected by certain effects. Moreover, it has been linked to eye damage, burns, and skin cancer. This reason alone is sufficient to ensure that proper protection covering is required when using it in industry. Several options are there, like adjustable portable systems to complete units for conveyor belts, small-sized equipment, and packaging materials (Bharti et al., 2022).

Cold plasma is a novel method for food contact surface decontamination. The most recognized sources are dielectric barrier discharge, atmospheric plasma jets, gliding arcs, and radiofrequency-based, and microwave-based discharges. Inherently, plasma is an ionized gas comprised of ions, free electrons, atoms, and molecules. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the most important active plasma agents for discharge to open-air atmospheres. Different species produced during cold plasma treatment include hydrogen peroxide, singlet oxygen, superoxide anion, peroxynitrite, dinitrogen tetroxide, dinitrogen pentoxide, nitrate, nitrite, and others. These created species are highly valued for their antimicrobial effects against a variety of microbiota (Nikmaram & Keener, 2022). However, there are still impediments to the actual large-scale implementation of this technology that must be addressed. One associated issue is that it has a commensurably lower impaling depth and therefore attenuated efficiency against surface biofilms. Therefore, more studies and knowledge are required to understand the inactivation mannerisms on a cellular level using more susceptive approaches. Another issue is that there are no standardized protocols for the decontamination of particular surfaces. However, it is an environmentally sustainable technology that does not require any chemicals nor yield any residues or wastewater. Moreover, lower energy requirements validate its efficiency/suitability for food contact surface application. Furthermore, design simplicity allows for adaptability for flexible and handheld applications, as well as uses in industry (Katsigiannis et al. 2022a, b).

Antimicrobial coatings for food contact surfaces are becoming increasingly popular. Because essential oils and botanical extracts derived from flora are natural in

origin, there is no concern about chemical toxicity or residues. These compounds have antimicrobial properties and have been used by mankind for centuries (Rossi et al., 2022). Extensive research on the potential applications in the food industry is currently underway. Several studies have confirmed the effective use of plant-based concoctions to food contact surfaces to ensure microbial safety. Essential oils are fancy composites which are cold pressed or distilled from botanical sources with the likes of stems, leaves, peels, etc. They are appreciably used in ancient times for numerous purposes and nowadays are popularized due to antimicrobial and antioxidant properties (Rudlong et al., 2022). Various reported action mechanisms underlying the compound's antimicrobial efficacy include microbial cell wall affecting the cytoplasmic membrane, cytoplasm congealing, membrane protein damage, and cell constituent leakage due to higher permeability (Torres Dominguez et al., 2019; Rossi et al., 2022). However, microbial inactivation and biofilm liberation depend on many factors such as the relationship between E.O.s effect and composition, concentration, involved bacteria, surface type, and surface smoothness (Nuță et al., 2021). Essential oils can be extracted from spices, herbs, fruits, vegetables, and their by-products, so this approach can alleviate consumer concerns about the green source.

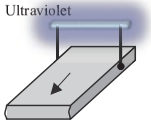
Table 16.3 provides an overview of the decontamination of food contact surfaces for the food industry, including potential benefits and associated concerns with various approaches. Growing cognizance towards incorporating the hurdle approach is high. Incorporating two or more technologies to ensure better safety and stability can be beneficial. Still, proper care must be taken to optimize the whole approach (Yuan et al., 2021). Schnabel et al. (2019) evaluated the synergic antimicrobial effect of plasma-processed air (PPA) and plasma-treated water (PTW) in a study. The plasma-on time (5–50 s) and the course of treatment length of the samples with PPA/PTW (1–5 min) were the determining factors in the unique and synergistic antibacterial capability of PPA and PTW. The only treatment with additive effects was 5 s + 1 min. Increased additive and inhibitory consequences were seen when the PPA treatment was prolonged to 50 s, followed by one, three, and 5 min. Amongst all tested combinations, inactivation was similar (additive) or enhanced (synergistic) compared to single treatments. For *B. atrophaeus* spores, single PAW and shorter CAP treatments showed <0.5 log CFU reductions, while given 3.2 log CFU reductions. The researchers promoted PTW as a potential alternative to efficient sanitation procedures in manufacturing plants by highlighting the combined advantages of PTW for washing and PPA for drying. In a recent study, Byun et al. (2022) pooled peroxyacetic acid or lactic acid with UV-C against *Salmonella* Enteritidis biofilms formed on different surfaces (stainless steel, silicone rubber, and ultra-high molecular weight polyethylene. The obtained results showed that biofilm on the contact surface significantly lowered ($P < 0.05$) by combined treatment of peroxyacetic acid or lactic acid with UV-C. In particular, PAA (50–500 μg/mL) with UV-C (5 and 10 min) reduced 3.10–6.41 log CFU/cm^2, and LA (0.5–2.0%) with UV-C (5 and 10 min) reduced 3.35–6.41 log CFU/cm^2 of *S. Enteritidis* biofilms on the surfaces.

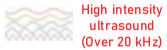
Table 16.3 An overview on technologies for decontamination of food contact surfaces

Technology	Exemplification	Treatment/type description	Advantages	Concerns	References
Thermal		Hot air Hot water	Cheap, environmentally friendly	The conventional method has limited efficacy, higher water consumption, and is unsuitable with heat and moisture-sensitive gadgetry.	Skåra and Rosnes (2016)
Chemical		Chlorine compounds such as hypochlorite, inorganic and organic chloramines, liquid chlorine, and chlorine dioxide can be used as a sanitizer to reduce and eliminate microbiota harbouring on food contact surfaces. Iodine compounds - Iodophors, alcohol-iodine solutions, and aqueous iodine solutions are commonly used to decontaminate surfaces. Ozone is composed of 3 O_2 atoms, is an oxidative agent that has the potential to inactivate microbes due to its oxidizing properties.	Represents a less expensive option than mechanical cleaning, effective as a process since all parts will be reached, resulting in more uniform cleaning.	Practically impossible with fully plugged equipment, severe damage can occur if improper procedures are applied, or unskilled personnel are employed in the application process.	Gallandat et al. (2021); Visconti et al. (2021)

(continued)

Table 16.3 (continued)

Technology	Exemplification	Treatment/type description	Advantages	Concerns	References
Physical	Ultraviolet 	U.V. radiation is specified as the portion of the electromagnetic spectrum between X-rays and visible light. U.V. light ranges from 100−400 nm, further subdividing into UVA (315–400 nm), UVB (280–315), and UVC (200–280), which is called as the germicidal range. Sources − Mercury lamps, excimer (E.L.), pulsed light (P.L.) and LEDs The use of U.V. radiation adds a variety of polar groups to the surface. Hence, this technique must add a specific functional group to the surface.	The process is very fast – with typical exposure times lasting only a few seconds. U.V. light is environmentally friendly.	U.V. light is known to be carcinogenic and causes a mutation in the body, and persistent exposure can lead to cancer.	Calle et al. (2021)
	Cold plasma 	This generates a wide range of reactive oxygen species (ROS) that are sufficient for the complete bacterial load in the semi-neutral plasma system, including bacterial spores and spoilage/pathogenic microorganisms. Sources − Dielectric barrier discharge (DBD), atmospheric plasma jets (APJs), gliding arcs (G.A.s), radiofrequency-based (R.F.), and microwave-based (M.W.) discharges	Rapid, waterless, zero contact, chemical-free	It cannot be used for the complete inactivation of endogenous enzymes that might be typically adherent to perishable processing units. Secondly, technology is limited to lab-scale only. Commercial-level coverage is still trivial.	Katsigiannis et al. (2022a, b)

	Ultrasound 	This process uses 'ultrasound' to safely enhance and intensify the cleaning process. This refers to sound waves with a frequency above the upper limits of human hearing. Regarding food contact surfaces, ultrasound is most often seen as an expedient for cleaning and removing biofilm.	Irregularities and pores at solid surfaces limit the effectivity; however, ultrasounds ably access and instigate deeper cleaning. Ultrasound also enhances the efficacy of chemical cleaning by favoring the release of contaminants such as oils, proteins, and even microbial biofilms, making them more accessible to chemicals	The ultrasonic field is variable and non-uniform throughout the treatment medium. The same levels of decontamination may not be achieved throughout the whole surface or material.	Astráin-Redín et al. (2019), Yu et al. (2020), Khaire et al. (2022), Dallagi et al. (2023)
Biological	Essential oils, extracts 	Eos, enzymes, biosurfactants, and extract formulations can be used as natural disinfectants to decontaminate food contact surfaces, thus lowering the risk of the indirect transfer of bacterial pathogens to food or persons.	The natural way is energy efficient, has no chemical involvement, antimicrobial activity, or insecticidal activity, and curbs down foul smells.	Accumulation of dead microorganisms on the surface effectively degrades the bactericidal property of the surface, also serving as a nutrient source for other microorganisms. Therefore, there is a need to release or remove the debris of dead microorganisms to maintain antimicrobial properties for the longer term.	Falcó et al. (2019), Rossi et al. (2022)

Validation of Contact Surface Cleanability and Disinfection as an Essential Component

Validation of the cleaning and disinfection process is an important step that can be added to the cleaning and production process. All of the determined prospects and applied regimes can only benefit if their proper application is made. To validate and authenticate the cleaning process, proper validation is necessary. Industries also manufacture foods for different community target groups, such as infants, pregnant women, people suffering from allergies, immunocompromised people, etc., which incites the need to validate the process properly. Moreover, proper cleaning inspection is required to maintain the brand image, particularly for products that are sensitive to people's religious sentiments (Schmitt & Moerman, 2016; Voss, 2018). ISO 22000 defines monitoring as "conducting a planned sequence of observations or measurements to assess whether control measures are operating as intended" and verification as "confirmation, through the provision of objective evidence, that specified requirements have been fulfilled" (ISO, 2005). Various steps are involved in the overall process, which emerges as an important part of the validation process regime. It includes everything from scope determination of cleaning validation to validation reports. The foremost step is to establish a validation objective, which can range from a proper cleaning check of the equipment to a particular microbial strain for a specific industry in order to substantiate the manufacturing authenticity. A qualified and experienced individual should be able to determine the objective and perform validatory checks (Schmitt & Moerman, 2016; Agüeria et al., 2021). When working on a decided objective, the first step is equipment qualification which requires proper certifiable proof of suitability for the intended application. Only properly serviced and operational devices should be marked for use Next is hazard evaluation, which is one the most important and critical steps in the procedure. HACCP is the foundation of the food safety management system that involves proper evaluation of risk factors that can compromise product safety. The evaluation consists of enumerating assessment factors that may have an impact on the cleaning results. Acceptance criteria should be determined on this basis. Therefore, if microorganisms or chemicals are present, whether the limit falls below the levels permitted by the legislative authorities for the particular industry must be determined. As an example, μg/cm^2 for organic matter or chemicals; while CFU (colony forming units)/cm^2 for microorganisms (Schmitt & Moerman, 2016).

This can only be accomplished through sampling followed by the proper procedures that will guide the success of the cleaning regimen. Both direct and indirect samplings are ideal, however, combined methods are most effective. The direct sampling technique involves collecting samples using swabs, wipes, sponges, or scraping devices (Agüeria et al., 2021). ISO 18593 provides detailed information about the procedure for sampling with swabs and contact plates (ISO, 2004). For an established amount of water from the rinse that can be captured and its leftover constituents identified, secondary sampling is more frequently performed. This technique is commonly used to sample inaccessible areas that cannot be easily

dismantled. Followed by analytical methods, which are important for detecting residuals and contaminants. Allergens, chemicals, and DNA can be found using techniques like serological and molecular biology tests, whereas product residues can be found using fast laboratory tests for ATP, which is proteins, or sugars. Besides chemical options, HPLCs are also available, but they are more costly alternatives. Any method can be used as long as it is validated, has a known limit of detection and quantification, and is sensitive enough to detect the established acceptable levels.

A suitable cleaning and/or disinfecting procedure should be selected and marked as an SOP (standard operating procedure). It must address the target microorganisms, cleaning frequency, equipment type, design, and anticipated food materials. A cleaning validation protocol must be established as a step. It is an imperative step as it outlines the entire process in detail, including the worst-case scenarios and corrective actions. There is also the report development, which summarizes the objective, course, evaluations, and results with specific commentary on the particulars (Holah et al., 2016; Ryther, 2014).

Additionally, maintaining a validated state is important and contributes to do the success of the process. This ensures the longevity of the validated conditions, though, necessary changes can be induced when required. Validation should be properly documented by qualified personnel and include the process for revalidation requirements. Overall, a food contact surface cleanliness and decontamination play a crucial part in ensuring safe, secure, and sound food manufacturing conditions.

Conclusion

Food safety is a grave concern for the food industries, customers, and federal agencies which are significantly affected by cross contamination of food products due to microbial contamination from the equipment surfaces. The primary cause of food product cross-contamination from contact surfaces is poor material selection and design for equipment construction, as well as ineffective cleaning procedures for installed equipment. Considering this, the major approaches that can be accustomed to minimize the microbial load from food contact materials are discussed in the chapter. The standards and guidelines on sanitary aspects of food contact materials are provided by different federal agencies, which helps to minimize food safety hazards that occur from contact surfaces. Future research should consider whether new approaches to ensuring food contact safety introduce any toxicological aspects to human health. Before moving forward, it is necessary to focus on the optimization of existing techniques to make them more effective. Additionally, combining a few techniques can have a positive impact on microbial safety and yield proficient results, with special consideration given to the economic feasibility of the approach. Finally, in light of the Covid-19 outbreak, future *in-vitro* studies should also look for the antiviral efficacies of the different technologies.

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Chapter 17
Film-Based Packaging for Food Safety and Preservation: Issues and Perspectives

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Introduction

Packaging is one of the last food processing operations at the manufacturing end and the first impression at the consumer end. The food packaging enables the containment in cost-effective manner with the quality and preservation of the food. The packaging of foods is advancing with technological advancements. The introduction of petrochemical polymers has impacted the food packaging industry to the extreme end. Almost in all the food and food processing sectors, synthetic polymeric or non-biodegradable packaging is introduced. The features such as flexibility, gaseous impermeable, strength, printability, and light weightiness have made it an efficient material for food packaging. In almost all the food sectors such as beverages, dairy, confectionary, meat, fruits, and vegetables, packaging plays a key role. Transportation, convenience, and the preservation of the foods are the major roles of a packaging display. The packaging designs and creation is determined according to the contents and the technology employed for the package. The correct variety of the packaging material determines the keeping quality of the contents (Francis et al., 2022). Commercially traditional food packaging materials such as metals, paper, paperboard, glass, etc. are partially or wholly replaced by plastic packaging. Plastic packaging comes in different forms such as PVC, PET, polystyrene (PS), polypropylene (PP), and polyamide (PA), mainly in rigid and flexible ones (Emblem, 2012). The concept of food packaging is evolving day by day,

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determined by consumer demand, market strategy, and product features. The new trends in packaging have not only revolutionized the food processing sector but also created a huge environmental hazard. The non-biodegradability and improper waste management made plastic a curse on the planet. Scientists and researchers are looking for the proper biodegradable technology and substation for petrochemical polymeric substances. Biopolymers of plant and animal origin are seen to have various film-forming properties (Diyana et al., 2021). The research is now to advance the commercialization and enhance the mechanical and barrier properties. The presence of biopolymers in nature is in abundance, which can act as a cheap raw material for packaging development (Ortega et al., 2022). The seaweed-based and agricultural waste are also utilized for the development of such biopolymers (Puglia et al., 2021). This can act as a cost-effective methodology to attain the goal of sustainability, safety, and waste utilization. The historical view on the use of such packaging started with the sausage casings. Nowadays different organic materials such as carbohydrates, proteins, and lipids are utilized for the development of the packaging (Wróblewska-Krepsztul et al., 2018). The films or coatings are made from carbohydrates, proteins, or lipids and can be directly applied to food products. To date carbohydrates are significantly developed for food packaging material for their ease of availability, biocompatibility, and polymeric property. Protein-based films are used in the pharmaceutical industry for capsule making (Gerna et al., 2023). Similarly, the gelation from the fish sources is exploited for food packaging at the lab scale as well (Rigueto et al., 2022). Cellulose is one of the most significantly used polymers to date for making wooden containers, but nowadays cellulose fibers are used for polymer development with molecular modifications (Aziz et al., 2022). Different types of papers are made by the utilization of cellulose from soft tissue to the toughest paper board. Different biopolymers from plant-based materials are now introduced as intact or primary packaging of food products. Such packaging is edible and can be consumed as part of food products (Chhikara & Kumar, 2021). It can be made from a variety of materials including starches, proteins, and polysaccharides, and it is often used as an eco-friendly alternative to traditional packaging materials such as plastic. Biopolymers reduce waste and environmental pollution by eliminating the need for disposable packaging. The quality and safety of food are maintained from external contamination and microorganisms. In marketing, it can add value to food products by providing an innovative and unique packaging solution (Agarwal et al., 2023). Several studies are going on to establish its integrity and effectiveness for the duration of the product's shelf life, and also for transportation and handling (Abdullah et al., 2022).

Despite certain challenges, there is growing interest in biodegradable film packaging as a potential solution to the problem of plastic waste and environmental pollution (la Fuente et al., 2023). Researchers and companies are exploring new materials and technologies to create safe, effective, and appealing edible packaging solutions that can help promote sustainability and reduce the environmental impact of food packaging.

Edible Coating

The edible coating is a layer provided on the fruit and vegetable artificially to give a barrier property to it and at the same time can be consumed with the food (Moeini et al., 2022). The important barrier properties are moisture barrier, oxygen barrier, and solute barrier properties. When moisture loss occurred from the fruit and vegetables it causes quality loss and oxygen diffusion cause oxidation, hence the deterioration of color, texture, and flavor (Chavan et al., 2023). Now these days' chemicals and synthetic materials are in use to increase the shelf life of fruit and vegetables. So, it is an initiative to replace synthetic material in the food sector. The edible coating and films are getting more attention due to the readily biodegradable nature and environmental consciousness of people (Kolybaba et al., 2006). There is also an advantage that if it is disposed of then it won't harm the environment. It also can carry some important attributes to the primary food like anti-oxidant properties, anti-microbial properties, vitamins, and nutrients (Robles-Sánchez et al., 2013). Other than these coating materials can carry flavoring agents, color enhancers, and taste improvers (Quezada Gallo et al., 2004). It can help in the fortification of food. There is a simple difference between coating and films. The coating is the direct application of coating material on the fruit or vegetable. It can be achieved by dipping, brushing, and spraying (Ganduri, 2020). But in the case of edible films preparation process is different. Continuous sheets are prepared and used as edible films on fruits and vegetables.

Classification of Edible Coating and Film

Edible film is formed by biopolymers which have other food grade plasticizer, additives to support its structural stability (Kaur et al., 2022). There are 3 types of edible films used for fruits and vegetable coating. Eg- Polysaccharide based, protein based, lipid based (Dhaka & Upadhyay, 2018). Each class of film-forming material provides different properties to the coating. These materials could be used alone or in combination with other materials to provide the required properties. All edible coating materials are formed from biopolymers only lipid-based edible coating materials are the exception. All edible coating materials are biodegradable in nature, which is consumer-friendly and environment-friendly also. It is very important to choose a perfect material or combination of materials to increase the shelf life of fruits and vegetables.

Polysaccharide Based Coating

Generally, polysaccharides are high in molecular weight and form hydrogen bonds when mixed with water, which makes them a good material for coating formation. Some polysaccharides are dissolved in cold water, some are in warm water and

some are even in neutral water (Zhang & Huang, 2022). Before making any film, it is necessary to check the mixing of the polysaccharides in the solvent. Some polysaccharide-based materials showed a huge improvement in the storage quality of fruits and vegetables, from which chitosan is a material which prevents microbial activity in the storage product (Fernandes et al., 2020).

Chitosan

It prevents the growth of Escheria coli and Staphylococus aureus, which are the major microbes in the food industry (Pavinatto et al., 2020). Not only just the antimicrobial property, but it also showed the ripening delay in some fruits and vegetables. It also promotes the actions like low respiration rate, low ethylene emission, and less weight loss (Pagno et al., 2018). Again, chitosan is a hydrophilic coating material, which made it a bad moisture barrier. That is why chitosan is used with other coating materials.

Aloe Vera

It is also come under polysaccharide-based coating material and with chitosan, it gives extra mechanical barrier and antifungal properties (Pinzon et al., 2020). Aloe vera also enhances its texture, taste, and visual properties. This is proved in the case of peach fruits (Hazrati et al., 2017). As it is a polysaccharide, it has hydrophilic nature too. So, using only aloe vera gel is not effective, so composite coating material is used to reduce the water vapor transfer. So, an additional layer of lipid should be used. It is also proved that the use of aloe vera gel with rosehip oil reduces ethylene production and moisture loss in plums (Martínez-Romero et al., 2017). It is also mentioned that the use of rosehip oil with aloe arborescence has a better effect on the firmness, total acidity, and weight loss of the fruit. The concentration of the lipid layer also plays a huge role. When the aloe vera gel is used with 0.1% sage essential oil on a tomato shows a better effect than the 0.5% sage essential oil (Tzortzakis et al., 2019). So, the proportion of the coating material is also important to reduce the respiration rate and ethylene emission. Aloe vera has generally less effect on respiration rate and weight loss but it helps to decrease the emission of ethylene. A similar result is shown by the composite coating of aloe vera, papaya leaf, and lemongrass leaf on papaya fruit (Lin & Zhao, 2007). The coating of aloe vera gel can increase the shelf life of papaya by up to 14.3 days when it is compared with non-coated papaya (Parven et al., 2020). Firmness is one of the important parameters in the case of a fruit, which shows consumer acceptance. It is affected by respiration rate and metabolic activity which causes ripening. In the case of mango, it is shown that the composite coating of aloe vera can increase the shelf life but the firmness gradually decreases (Ebrahimi & Rastegar, 2020).

Alginate

It is also known as sodium alginate. It is generally extracted from brown seaweed species and used as an edible coating on food material. It is available in the form of salts (Sarkar & Maity, 2023). It needed a conversion process from salt form to sodium alginate form. Alginate is the good barrier of oxygen, but fails to prevent moisture loss like chitosan. It has experimented that in the case of pansies there is no significant difference between the water losses in cold storage (Fernandes et al., 2018). But using sodium alginate with other compounds enhances its properties also. The extract of Ficus hirta enhances the antifungal properties of sodium alginate. Again, it shows a greater level of antioxidants in Nanfeng mandarin than the only use of sodium alginate coating (Chen et al., 2016). In blueberry fruit, the alginate-coated sample incorporated with pectin shows development of yeast after 10 days of storage whereas the control sample shows after 2–4 days of storage (Mannozzi et al., 2017). The red color of sweet cherry shows the quality of the fruit. Alginate-coated sweet cherry shows less change in color and phenolic compounds during storage (Díaz-Mula et al., 2012). There is also evidence that using 2% sodium alginate incorporated with 1% grape seed extract can decrease the fungal decay in grapefruit (Aloui et al., 2014). It can decrease the weight loss by about 25% compared with the uncoated control samples. Alginate forms a thick layer on the fruit and vegetables. So that it also helps to preserve freshly cut fruits as it is vulnerable to rapid decay. Freshly cut watermelon coated with alginate shows a result of less weight loss and preserving the texture for up to 13 days (Sipahi et al., 2013). Again, the fruits like peach which ripe faster than other fruits needed to delay the ripening process. Alginate incorporated with salicylic acid delay the post-harvest ripening and maintain the phytochemical concentration (Bal, 2019).

Pectin

It is an anionic polysaccharide material, unlike chitosan. It makes a perfect Nano multilayer on the cationic polysaccharide like chitosan. Due to its hydrophilic nature, its water loss is more compared to other coating materials but still less than the non-coated fruit materials (Huang et al., 2021). But when pectin is incorporated with chitosan it shows good gas barrier properties. In the case of mangoes, it results in a decrease in oxygen permeation which delay the ripening (Bartolomeu et al., 2012). But when the fruit is highly perishable due to respiration and vulnerable to weight loss pectin and chitosan show a significant improvement than non-coated fruits. Even it conserves the firmness of it. It helps to increase the shelf-life of strawberries from 6 to 15 days (Treviño-Garza et al., 2015). Not only just the whole fruits, pectin can also improve the shelf-life and sensory characteristics of fresh-cut fruits. Incorporation of calcium lactate shows a shelf life of fresh-cut watermelon up to 14 days which was normally 9 days (Ferrari et al., 2013). But sometimes it decreases the sensory acceptance score as it dilutes the liquid directly. Again, the thinner-coated fruits and vegetables show a greater acceptance and hence get more sensory scores (Martiñon et al., 2014).

Protein-Based Coating Material

Protein can be classified as water-soluble protein and water-insoluble protein. Fibrous proteins are generally insoluble proteins (Sim et al., 2021). As the amino acids present in insoluble proteins are connected with each other with hydrogen bonds. It is the main reason that protein forms fiber and at the same time insoluble in water. It was found that a number of proteins can be dissolved in water, ethanol, and methanol (Bromberg & Klibanov, 1995). So, water and ethanol or their solution can be used as protein carriers in the coating solution. Before using protein in an edible coating, it should be denatured. Due to this process, more sites will be opened to form bonds which will give a cohesive nature to the coating material. Protein is a complex compound that is made of different amino acids. The characteristic of protein-based coating material depends on the nature of amino acids whether it polar or non-polar. On the basis of polarity, the coating material shows a hydrophilic or hydrophobic characteristic. Proteins rich in alanine and leucine create a hydrophobic nature of the protein. Again, protein is categorized under animal source (Casein, whey protein, meat protein, Egg albumin, Keratin protein) and plant source (Soy protein, Corn zein, Wheat protein, cottonseed protein, peanut protein) (Zaritzky, 2011). Some of them are already in use as an edible coating and biodegradable film. Some of them are under research.

Whey Protein

It is generally extracted from milk when sodium caseinate is condensed during cheese making. Like polysaccharide-based coating material, it needs a plasticizer to make the layer more flexible. Without a plasticizer, there is a crack formation on the surface (Song et al., 2022). Whey protein can be a good barrier for gasses if it is used with certain plasticizers. The combination of whey protein isolates and glycerol act as a bad barrier of gases which increases the respiration process (Lerdthanangkul & Krochta, 1996). Hence the ripening process is faster. It has been seen that the use of sorbitol in place of glycerol has a far better barrier effect on gas and water vapor (Chen, 1995). The protein shows amphiphilic nature means it shows both hydrophilic and hydrophobic nature. Hydrophobic nature is the best to protect the primary food from water vapor. The lipid layer is used on the protein layer to prevent it from water vapor. The concentration of whey protein plays a significant role in a barrier property. More the concentration better the barrier properties (Javanmard et al., 2013). Bee wax can also be used to enhance the water vapor barrier of whey protein. It is experimented that 20% Bee wax is better for the edible coating (Soazo et al., 2015).

Soy Protein

It is a product from soybean, which is generally used as a substitute for animal protein. The protein content present in soybean can reach as high as 50%. So, it is a rich source of protein. Besides direct consumption, soy protein can be used as an edible coating material for fruits and vegetables (Momin et al., 2021). Like polysaccharides, it is used with lipid material to improve the moisture barrier property of the material. The main parameters of an edible coating are barrier properties, mechanical properties, and appearance after coating. Experiments have done with 3–5% soy protein isolate 1% olive oil, and 0.40% hydroxypropyl methylcellulose (Nandane et al., 2017). It is applied on pear fruit and results are better than uncoated fruit material. Soy protein isolate is a good barrier of oxygen so it decreases the respiration process and hence decreases the ripening. In phalsa fruit, it is observed that the TSS concentration is low and moisture loss is less in the case of coated samples. The shelf life is increased by 3–5 days than a controlled sample (Dave et al., 2016). Again, in the case of citrus fruit, blue mold is a severe issue now these days. But the addition of antimicrobial agents like limonene in soy protein isolate shows a significant reduction of mold in Persian lime (González-Estrada et al., 2017). As soy protein is responsible to reduce the respiration of the fruit, the incorporation of antioxidant properties will help to reduce the enzymatic browning of fresh-cut fruits. Ferulic acid is added to soy protein and experimented on fresh-cut apples. It decreases the water transpiration rate and enzymatic browning of apples (Alves et al., 2017). There is a recent development in the area of nanocomposite edible films to enhance the properties of coating material. The use of silicon dioxide nanoparticles in soy protein isolate decreases the oxygen permeation even further. It is observed that it enhances the tensile strength of the coating material on apples (Liu et al., 2017).

Gelatin Films

It is a water-soluble protein derived from animal sources. The film prepared from gelatin does not impart a pungent smell. Initially, pig skin was used to produce the gelatin but now other vertebrate animals are in use to produce it. The basic process to extract the gelatin protein is the hydrolysis of insoluble protein present in skin bone and connective tissue (Usman et al., 2022). Gelatin has a triple helix structure and at a very low temperature, it can form a gel in comparison to other film-forming materials. As gelatin is a hydrophilic material, it is more susceptible to water vapor transpiration. But in combination with corn zein protein material, it performed a better result. Other than that transparency, mechanical strength of the coated material, and UV barrier properties were improved (Xia et al., 2019).

Corn Zein Protein

It is an insoluble protein. The hydrophobic property of corn zein protein is due to the amino acids present in it being non-polar in nature (Tadele et al., 2023). Due to its hydrophobic nature, it could be a better biodegradable film material in the near future. As it shows a non-polar nature it is soluble in ethanol solution and film formation is relatively easy. But plasticizers like glycerol are necessary to prevent cracking after film preparation.

Lipid-Based Coating Material

Lipid is generally used as a protective coating. Lipid is hydrophobic in nature so it is used to prevent moisture transportation from the fruit and vegetables to the environment (Milani & Nemati, 2022). The hydrophobic nature of lipids is due to the low polarity of it. So due to this lipid forms weak mechanical structure. Sometimes it is thicker and brittle in nature. Glycerides, waxes, and resins come under this category. So, it is used in the composite film. More commonly it is used with polysaccharide which provides better mechanical strength (Yousuf et al., 2022).

Waxes

These are more common in the coating sector. The various waxes are carnauba wax, paraffin wax, bee wax, and candelilla wax. Carnauba is plant-based wax. It is collected from palm tree leaves and has the highest melting point of all the naturally occurring waxes (Devi et al., 2022). Due to its high specific gravity, it is used with other waxes to increase the boing point. It gives more mechanical strength than other waxes. It is observed that the use of carnauba wax doesn't prevent the respiration rate but it significantly decreases the water vapor transportation (Chiumarelli & Hubinger, 2014). Carnauba wax is a GRAS (Generally recognized as safe) substance and is allowed to use as a coating material on fruit and vegetables. Paraffin wax is generally extracted from crude petroleum. Unlike carnauba wax, paraffin wax is not a GRAS substance. It is obvious because synthetic paraffin wax is not allowed in all countries. Any waxes whose melting point temperature is higher than the boiling point of water can cause severe problems during coating. If there is free water on the surface, it can form air pockets which can later decrease the shelf life of the product. Paraffin wax is preferable to root crops and sugarcane. Bee wax which is also called white wax is produced directly from bees. After the extraction of honey, a refining process is followed to get the bee wax. Bee wax comes under animal-based waxes and also comes under GRAS substances. It was also observed that starch incorporated with beeswax can increase the shelf life of products with low water vapor transport rate (Oliveira et al., 2018). Candeilla wax is a plant-based wax like carnauba wax. The hardness of the wax is lesser than the carnauba wax but

more than beeswax. The setting time of the wax is more than other waxes. This wax also comes under GRAS substances.

Resins

These are however similar to waxes. It is secreted generally from a special duct called resin duct from a plant. It happens when the plant is affected by an injury or infection. Resin can also be prepared synthetically. Polyvinyl, polystyrene, polyethylene, polyesters, and epoxy resins, etc. are the synthetically prepared resin used in plastic industries (Ibrahim et al., 2021).

Shellac resin is derived from the secretions of the female lac bug, Laccifer lacca. It possesses the unique property of being soluble in alcohol, with ethanol commonly used for obtaining liquid shellac. Alternatively, shellac resin can be dissolved in alkaline solutions. It is important to note that shellac resin is classified as generally recognized as safe (GRAS) substance, which multifold its application in food and related industries, subject to regulatory considerations (Kumar et al., 2023). Another type of commonly used wax is Candelilla wax (CW), made from the leaves of Euphorbia cerifera and Euphorbia antisyphilitica, two tiny shrubs that are endemic to northern Mexico and the southwestern United States. CW is a widely used, FDA-approved food ingredient that is primarily employed as a glazing agent and binder for chewing gums (Aranda-Ledesma et al., 2022). Candelilla is effective against water vapor transmission thus possess potential functionality as a constituent of different food formulations. But when shellac resin is incorporated in candelilla wax, it gives a firm structure, gloss, and lesser moisture loss properties to the product (Alleyne & Hagenmaier, 2000).

The technology of edible coating and films is creating a new path for tackling plastic-based packaging material. It can be used in new-generation packaging material as it can impart a wide range of properties. Three major category products are used to prepare coating material e.g., Hydrocolloid, lipid, and composite of both. Hydrocolloid is a good barrier to oxygen, and carbon dioxide but not to water vapor. As lipid is hydrophobic in nature in combination with hydrocolloid, it could be a better solution to water vapor transmission. Hydrocolloid imparts good mechanical strength to coated material. Other than that, all these materials can be used as a food fortification medium with water-soluble vitamins, minerals, and antioxidants. More research is needed in the edible coating and film sector to improve the film preparation techniques and their properties.

Application of Edible Films in Food

The applicability of edible films is growing at a rapid rate, to overcome environmental and food packaging issues. Researchers are continuously exploiting new means of development and different formulations to develop the best compatible

packaging material. Edible films provide a protective barrier and enhance the shelf life of various food products. Film-based packaging material helps to maintain the quality and freshness of food products by providing a protective barrier against moisture, oxygen, and other environmental factors that can cause spoilage (Aga et al., 2021). Film-based packaging provides an innovative and sustainable packaging solution to differentiate products and promote environmental sustainability. One of the notable applicability of the films is their usage in the packaging of fresh produce. The quality and shelf-life of fruits and are vegetables extended by providing a protective covering around the commodity. Various researchers have suggested the use of films in maintaining the quality of meat and poultry, thus reducing the problems of contamination. Also, the studies reveal the antimicrobial properties of certain functional films that inhibit microbial growth in various products (Dharini et al., 2022). The organoleptic characteristics of certain products are enhanced by wrapping them in functional film. The film induces the desirable characteristics for the marketability and consumption of the product. Some moisture-desirable products are protected by wrapping in a water barrier film around the product.

The other noteworthy application of film-based packaging is its use as sustainable food packaging material. The use of various renewable and biopolymers to develop the film-based packaging films drastically reduce the usage of petrochemical polymers, thus reducing waste production and environmental problems. Agricultural waste and seaweed are generally exploited for the making of such films. The film-based materials for packaging applications are going through many processing procedures and ingredient incorporation. The main aim of such packaging is to address the challenges of sustainability, protection, containment, transportation, and shelf life. Therefore, the packaging film development is still in its primary phases. More of studies and research work needs to be carried out to improve and diversify its applicability in the packaging sector. In addition to sustainability, films can be utilized as a carrier for antimicrobial chemicals, control oxygen and carbon dioxide exchange rates, and prevent microbiological deterioration. The properties such as flexibility, transparency, and ability to protect products from external factors such as moisture and air make films suitable for packaging of bakery products. Bakery products such as bread, cakes, cookies, and pastries are often packaged using film packaging, which can be made from a variety of materials including plastic, biodegradable materials, and paper. Film-based packaging provides a protective barrier that can help to prevent moisture loss and keep products fresh for longer periods of time. This is particularly important for bakery products that are prone to staling or drying out quickly, such as bread and pastries. The protection from external factors is facilitated by the use of film packaging material. This will help to preserve the aroma and flavors of bakery products, making them more appealing to consumers. Also, the film-based packaging material provides a convenient and hygienic option for consumers. Film packaging is also a continent for opening and closing of the package, allowing consumers to access the product without compromising its freshness or quality. Additionally, film packaging can help to prevent contamination by providing a protective barrier between the product and the external environment.

Visual appearance of bakery products is also enhanced by film wrapping, clear film packaging can showcase the product inside, allowing consumers to see the product before purchasing it (Simmonds & Spence, 2017). Biodegradable films, such as those made from plant-based materials like corn starch, are also gaining popularity due to their eco-friendliness and ability to break down naturally in the environment. Paper-based films, such as parchment paper and wax paper, can also be used to package bakery products, providing a natural and sustainable option. Film packaging is a versatile and effective option for packaging bakery products.

Film-based packaging finds its scope in the dairy industry as well for packaging of products such as milk, cheese, yogurt, and butter. The use of film-based packaging provides several benefits, including product protection, extended shelf life, and ease of use, a barrier against oxygen, moisture, and light. This protection helps to preserve the quality, texture, and flavor of dairy products, ensuring that they arrive at the consumer in optimal condition (Thakur & Raposo, 2023). Film-based packaging prevents the growth of bacteria and other microorganisms, thus extending the shelf life of dairy products. This is particularly important for perishable dairy products such as milk and cheese, which can spoil quickly if not stored properly. Biodegradable films, made from materials such as corn starch or polylactic acid, are gaining popularity due to their eco-friendliness and ability to break down naturally in the environment. Paper-based films, such as wax paper and parchment paper, can also be used to package dairy products, providing a more natural and sustainable option. In addition to traditional film-based packaging, there are also innovative packaging solutions such as, active packaging, this type of packaging can include oxygen scavengers, antimicrobial agents, and moisture absorbers, all of which can help to maintain the quality and freshness of dairy products. Film-based packaging also helps to prevent the contamination of meat products. The films provide a barrier against external contaminants, such as dust, insects, and other pollutants, that can contaminate the meat and compromise its safety. In the meat industry, it can be used for packaging various meat products, including beef, pork, poultry, and seafood. There are many advantages to using film-based packaging for meat products, such as extending shelf life, preserving freshness, and preventing contamination. It also imparts the freshness of the product by reducing desiccation and discoloration. It also helps in the retention of the original flavor of meat products and does not confer the sensory qualities. Active films are nowadays an interesting field in achieving desirable characteristics of meat products.

For the high-quality food product with extended shelf life, some functional ingredients are loaded inside a film matrix. These functional ingredients provide necessary protection from any sort of biological, chemical, and physical hazards. Organic or inorganic functional ingredients such as extracts, essential oils, nanoparticles, and metal oxides provide essential functional properties (Vieira et al., 2022). These can act as potential antimicrobials and antioxidants in films, wrapped in food products.

Safety Concerns of Edible Films

The application of edible films in food offers a promising solution for enhancing the quality, safety, and sustainability of food products. From packaging fresh produce to creating functional coatings and barriers, edible films can provide a range of benefits for food manufacturers, retailers, and consumers. The safety aspect is also another challenge to work on. The films need to be validated and certified by the national and international agencies for their safety as food grade. The materials used in edible films may contain chemical additives, such as plasticizers, colorants, and preservatives. These additives can potentially migrate into the food product and cause chemical contamination. The ingredients of the film should not confer the chemical, or biological nature of food. There should be an in-depth study of ingredients that are to be used in food packaging. Moreover, the composition of the particular ingredient should not cross the maximum permissible limits. The main safety concerns associated with edible films need to be addressed to make them fit for food packaging. Edible films can be made from a variety of materials, including proteins derived from animal or plant sources. This can lead to potential allergy concerns for individuals who are allergic to specific proteins or ingredients. Manufacturers need to be transparent about the ingredients used in their edible films and provide clear labelling for consumers. Edible films can provide a suitable environment for the growth of microorganisms, particularly if they are not stored and handled properly. This can lead to the potential for microbial contamination of the food product, which can cause foodborne illness. The edible films should be produced and stored in a hygienic environment and should have adequate barriers against microbial contamination. Safe and approved additives should be used in film development that should not cause chemical contamination of the food product. In some cases, edible films may not dissolve or break down completely in the mouth, leading to the potential for choking hazards. Edible films should dissolve or break down easily in the mouth to ensure their safety for consumption. Some edible films may contain ingredients that interfere with nutrient absorption in the body. For example, some edible films made from chitosan have been shown to bind with dietary fats, potentially reducing the absorption of important nutrients such as vitamins and minerals. Therefore, edible films do not interfere with nutrient absorption, and they are safe for consumption.

Future Scope

Consumers and businesses alike are increasingly aware of the environmental impact of traditional packaging materials and are seeking more sustainable alternatives. Film-based biodegradable packaging solution has emerged as a promising alternative to petroleum-derived polymers. Although the barrier properties of biodegradable films are far less than the petrochemical polymers, still the biopolymers offer a suitable substitution. There are many emerging solutions for biodegradable

packaging, including plant-based materials, bioplastics, edible packaging, algae-based materials, and paper-based materials. As research and development in sustainable packaging continue, more innovative and eco-friendly biodegradable packaging solutions will be available. Researchers are working on developing advanced materials that are more durable, flexible, and cost-effective than current biodegradable materials. These materials may include bio-based polymers, nanocellulose, and other advanced materials that can be used to create packaging with improved performance and environmental benefits. Recent advances in packaging are active and intelligent packaging films. Some novel strategies for the fabrication of such polymers that are often used these days are composite, multilayer, and emulsified films. Also, some advanced methods are also employed for the development of nanocomposite films. 3D printing technology is being explored as a potential way to manufacture biodegradable packaging. This technology can create complex and customizable shapes, which can be tailored to specific products and reduce waste. 3D printed biodegradable packaging could also be made from a variety of sustainable materials, including plant-based materials and bioplastics. Active packaging is also an emerging technique in film development. It helps in the shelf-life extension of food products, reducing food waste and the need for additional packaging. These additives can include oxygen scavengers, antimicrobial agents, and moisture absorbers, all of which can help to maintain the quality and freshness of food products. Advanced materials, 3D printing, active packaging, blockchain technology, and the circular economy are all areas where biodegradable packaging can make a significant impact in reducing waste and improving sustainability.

Conclusion

From extending the shelf life of products to enhancing their visual appeal, film packaging offers a range of benefits for manufacturers and consumers. Also, the food sector is emphasizing biopolymers usage as packaging materials because they are safe and well adapted to include active agents (such as emitters and scavengers) that preserve food quality and extend shelf life. The barrier and mechanical properties need to be improved to compete with synthetic polymers. As research and development in sustainable packaging continue, we can expect to see even more innovative and eco-friendly film packaging solutions for the industry.

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Chapter 18
Potential Use of Biotechnological Tools to Eradicate Microbial Biofilms

Parul and Ajay Pratap Singh

Introduction

Microbial biofilms are aggregates of microorganisms embedded in autogenic extracellular network of proteins and exopolysaccharide materials that adhere to an abiotic or biotic surface. Biofilms are commonly known as the city of microbes and follow the unique pattern of growth to achieve the higher level of organization of free-living microbes. According to Donlan and Costerton (2002) "Biofilm as a structured community of microbial cells enclosed in a self-produced polymeric matrix and adherent to a surface to interface, and to each other" still remains the most appreciated definition of biofilms (Mishra et al., 2020).

Basically, microbial biofilms are complex, dynamic and three-dimensional heterogeneous structures in which cells are interconnected by Extracellular Polymeric Substances (EPS). EPS are a blend of polysaccharides, peptides, nucleic acid and other substances produced by microorganism itself. EPS provides protection to microbial cells under adverse environmental conditions thus encasement acts as a house for cells. Biofilms can withstand metal toxicity, ultraviolet light, lethal effect of antimicrobials and other chemical agents like soaps, detergents, disinfectant and other cleaning agents (Rodríguez-Lázaro et al., 2018).

Nowadays biofilms are a big issue for the food industry, medical field, naval and other industries also. Certain microbes have the capability to aggregate over various surfaces of materials and clinical devices such as medical implants, prosthetic implants, catheters, sutures, intrauterine devices and contact lenses to produce the biofilm.

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In the food industry, biofilm forming food borne pathogens may contaminate the raw material and food products through secretion and excretion of toxins and enzymes that may create risk for consumer's health. Similarly, medical devices and implantations are also attacked by biofilm forming pathogens that further lead to infections in the human body. Water supply networks are also affected by biofilms that lead to contamination of water, deterioration of water quality and corrosion of water channels by these microbes (Ramirez-Mora et al., 2018). Biofilms are the state in the life cycle of microbes that enhance the attributes of resistance against external attack of antibiotics, chemicals and disinfectants. Future insight the immense need to implicate effective methods for elimination of biofilms from the environment.

Biofilm Formation

Biofilm formation by planktonic cells (free living cells) over a surface is a natural process by which free living microbes attach and aggregate to surfaces and grow into multicellular communities. It is a series of complex process and accomplished mainly in five stages:

- Reversible attachment
- Irreversible attachment or colonization,
- Proliferation
- Maturation
- Dispersion

In this process the life cycle of microbes changed from unicellular to multicellular or planktonic to sessile and this transition between two stages leads to the formation of biofilms. Usually biofilms comprise 10% of dry mass that represent microorganisms while the rest 90% derived from the matrix of biofilm. Indulgent microorganisms in biofilms categorized it as monospecies (formed by a single microorganism) or multispecies (two or more than two types of microorganism) (Satpathy et al., 2016).

The stages of bacterial biofilm formation are given below

Reversible Attachment

The fundamental process in the growth of biofilm begins with reversible adherence of microbial cells to surfaces. It is a complex process and mediated by a series of physical and chemical interactions. Certain surface attributes like surface conditioning, net charge on substrate, hydrophobic surface, surface irregularities, and growth conditions play crucial roles during attachment of bacterial cells. Certain conditions favor the reversible attachment of cells that is attained through delicate interactions

such as Vander Waals and electrostatic forces. The presence of wall and membrane teichoic acid in Gram-positive bacteria and outer membrane phospholipids in Gram-negative bacteria results in a net negative surface charge on the majority of bacterial cells at neutral pH. As a result of charge repulsion, negatively charged substrates inhibit bacterial adhesion, whereas positively charged surfaces promote bacterial attachment and the subsequent formation of biofilm (Verderosa et al., 2019). Finally balancing between attractive forces and repulsive forces determine the attachment of bacterial cell surface over substrate. Secondly, during reversible attachment bacteria usually remain in a random brownian motion leading to detachment of the cell from the surface. The ensuing forces of attraction and repulsion encourage reversible bacterial adhesion to the surface.

Bacteria's ability to sense the abiotic and biotic substratum is facilitated by bacterial appendages that allow them to adhere and form a biofilm. Surface Interaction of flagellar motors triggers a signal cascade that selectively regulates the flagellum biosynthesis pathway while expression of genes that regulate biofilm formation is upregulated. Apart from surface characteristics several other physiochemical factors can influence bacterial biofilm formation such as environmental temperature, osmolarity, pH, nutrient abundance and bacterial cell density. These variables may alter the surface characteristics of both bacteria and the substratum, which would affect bacteria's capacity to adhere to solid surfaces. (Zhang et al., 2015).

Irreversible Attachment or Colonization

In the immediate aftermath of the reversible phase of adhesion, bacteria begin secreting an exopolysaccharide substance, which initiates the irreversible phase of the synthesis of the biofilm matrix. (Abdallah et al., 2014). The EPS matrix's core constituents include a variety of macromolecules such as protein complexes, nucleic acids, lipids, and polysaccharides. During the irreversible attachment forces are stronger to bind bacterial cells through the surface. Bacterial outer membrane proteins, lipopolysaccharides, flagella, and surface adhesions such as fimbraial (including curli and pili) and a fimbrial adhesins mediate irreversible attachment (Srinivasan et al., 2021). The different physical forces and chemical bonding such as hydrogen or covalent bonding as well as electrostatic, ionic, and hydrophobic interactions are also involved in this process. The EPS secretion by bacterial cells is further regulated by quorum sensing mechanism. Bacterial cells aggregate on solid surfaces via intercellular cohesion, whereas their attachment to biotic and abiotic surfaces is mediated by hydrophobic and ionic interactions. (Costa et al., 2018). The bacterial secondary messenger cyclic diguanosine-monophosphate (c-di-GMP) signalling pathway regulates the cellular process responsible for the transition from reversible to irreversible biofilm formation (Toyofuku et al., 2016).

Biofilm Proliferation

After the irreversible attachment of bacterial cells over the surface process of proliferation commences. In this phase cells multiply either by binary fission or asymmetric division (Laventie et al., 2019). Proliferation of cells triggers the intercellular communication, activation of secondary messengers and production of EPS. Formation of microcolonies initiated by attachment of bacterial cells to the surface as well one another by secreting microbial EPS that entraps the cells. The enormous productions of EPS lead to formation of multi-layered structure that gradually transformed to 3D structure of bacterial biofilm.

Biofilm Maturation

Process of maturation started after the formation of micro colonies or immature biofilms. Further, cells are aggregating over the micro colonies to form the macro colonies. Intensive cell proliferation and EPS production continues until biofilm acquires an optimal cell density. During the maturation phase, intra-colony channels within the biofilm matrix facilitate the influx of nutrients, oxygen, and various other elements indispensable to bacterial growth, as well as the efflux of waste products and dead cells. Intercellular communication is strong and mainly carried out through quorum sensing. EPS is a multi-layered, three-dimensional bacterial cell structure that accounts for more than 90% of the dry mass in mature biofilms.

Dispersal/Detachment

Detachment or dispersal of biofilms is the end phase of the formation process. After maturation, bacterial cells start to leave the old house and spread over new stratum to form other biofilms. Thus the cycle of biofilm formation is going on in nature to maintain itself. Detachment of microbial cells is a natural and complex process that is influenced by a number of intrinsic and extrinsic factors. It was found that various intrinsic and extrinsic factors like EPS degrading enzymes, nutritional deficiency, mechanical shear forces and environmental factors like temperature, pH, dissolve oxygen can influence biofilm dispersal (Gupta et al., 2016). On the basis of the causal factor of dispersion, it may be of two types either active or passive.

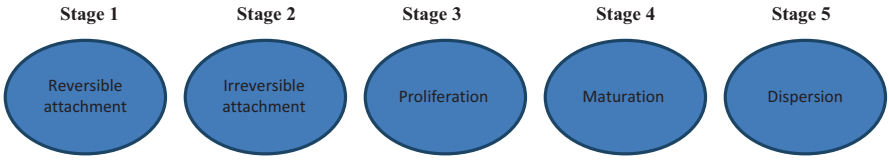
Active Dispersal

In active dispersal immediate dispersal of microbial cells occurs to combat the intrinsic factors like low amount of EPS, nutritional and oxygen deficiency in the internal environment of biofilms. Large number of microbial cells start to slough off

from the center of biofilms to create a hollow cavity inside the three dimensional structure. Active dispersal, a gene regulated mechanism, governed the cell motility by up regulating genes to increase the synthesis of locomotor organs like flagella. The increased movement of bacterial cells inside the biofilm enhances the dispersion. In contrast, genes down regulate the production of EPS and synthesis of attachment appendages like fimbriae to create the instability in the internal environment of biofilm that also favors the active dispersal. The signalling pathway c-di-GMP additionally takes part in the dispersal process; the low concentration of c-di-GMP promotes the detachment of cells. In microbial cells, low levels of oxygen and high levels of glucose diminishes the intracellular level of c-di-GMP and in turn enhances the dispersal process (Kostakioti et al., 2013).

Passive Dispersal

In passive dispersal, release of small portions of bacterial cells under the influence of mechanical shearing force from the biofilm take place.



Stages of biofilm formation

Components of Biofilms

The main constituents of microbial biofilms are microbes themselves, extracellular polymeric substances secreted by microbes, water containing structures inside the matrix pores and channels and extracellular DNA.

Microbial Cells

Microbial cells are the main players that form the biofilms. Among the microbes, bacterial cells have the special capability to adhere on the surface and produce the biofilm. Different bacterial genus likes*Pseudomonas, S. aureus, Listeria* and *E. coli* varied in potential to adhere on the surface and to produce biofilms. External appendages over the surface of bacteria like pilli, fimbriae and flagella are important organelles that facilitate biofilm formation.

Extra Cellular Polymeric Substances (EPS)

Extracellular polymeric substances (EPS) also known as extracellular matrix (ECM) produced by microbial cells and also embedded themselves to acquire the protection from adverse conditions. Equity of EPS in biofilm varies and ranges from 90–99% of dry mass of biofilms. EPS production capability also varies from microbe to microbe. EPS differs in its composition, formation and structure and variations are usually due to type of bacterial species and its surrounding environment. Biomolecules like polysaccharides, proteins, lipids and extracellular DNAs (eDNA) are the main constituents of EPS and among these major ones are polysaccharides. Protein part of EPS comprises enzymes and external appendages like fimbriae and pili. In Gram-positive bacteria polysaccharides are mainly cationic while neutral or polyanionic in Gram-negative bacteria (Flemming et al., 2016).

The main function of EPS is to ensure protection to microbial cells and alongside rigidity to 3D biofilm structure. Thus, physical functions are adhesion, cohesion, stability and scaffolding. EPS act as defensive layer for microbial cells against natural and synthetic antibiofilm agents. Important one listed as frequently used disinfectants, sanitizers and antimicrobials in food processing plants. Inspite of protection other requirements like availability of nutrients, quorum sensing and conducive environment facilitated for microbial cells (Costa et al., 2018).

Water Filled Structures

Channels and pores are water-filled structures in a matrix of biofilm. Channels are long and relatively narrow structures connecting two places to facilitate transport and in line also known as "rudimentary circulation systems in biofilms" while pores can serve as storage and buffering pools and are distinguished from channels (Quan et al., 2022). Channel and pore development and function are governed by fundamentally separate systems, and both may be differentiated according to their formation process, functionality, and dimensions. Main function of channels is to allow transport of nutrients, signalling molecules, biomolecules, antimicrobials and waste products.

Extracellular DNA (e DNA)

The eDNA in biofilms is an important component and it provides the structural stability to 3D structure of biofilms. Simultaneously, it promotes the EPS production and gene transfer through transformation. Number of bacteria like *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*,*Staphylococcus* species (*S. aureus, S. epidermis*), *Enterococcus faecalis*, *Helicobacter pylori, and Campylobacter jejuni* release eDNA in their biofilm (Yin et al., 2019).

Factors Affecting Biofilm Formation

Various intrinsic and extrinsic factors affect the biofilm formation on abiotic and biotic surfaces. Factors like temperature, nutrient availability, oxygen tension, alkalinity and the physicochemical properties of the substratum of surface, especially texture and hydrophobicity influenced the process of aggregation of cells to form biofilms.

Sectors Affected by Microbial Biofilms

Microbial Biofilms in Food Industry

Foodborne pathogen forms the biofilm on surfaces contacting with foods. Data revealed that more than 60% foodborne outbreaks are related to biofilm forming microbes. Biofilms seems to be a great challenge in food industry especially dairy sector. Environmental contaminants, food handlers and food processing plants are the main source of food borne pathogens over the contact surfaces. Remnants of food attract the microbes and provide the nutrients for multiplication and promote the biofilm formation. Matured biofilms act as continuous source of pathogen that may lead to food spoilage and risk to consumers health. In food industry list of common biofilm-forming food borne pathogen and spoiling organism include *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* O157:H7, *Pseudomonas* spp., *Vibrio parahaemolyticus, Clostridium perfringens*, *Campylobacter jejuni*, *S. aureus*, *Shewanella putrefaciens, Cronobacter* spp., *Geobacillus stearothermophilus*. These microbes either produced monospecies or multispecies biofilms, however multispecies are more common and more difficult to eradicate (Berlanga & Guerrero, 2016). Inspite of food spoilage and food poisoning to consumers biofilms cause damage to equipment surfaces of food processing plants by corrosion. it also reduces the production efficiency by increasing the fluid frictional resistance to surfaces may decrease heat transfer across the equipment. Thus, biofilms in food industry are a big challenge to consumers, food products and processing plants also.

Microbial Biofilms in Medical Field

In the medical field biofilms observed inside the living tissue of human body (teeth, ear and lungs etc), dead tissues and on medical devices (catheter, transplantation devices, contact lenses, prosthetic heart valves, stents, pacemakers, shunts and artificial joints or limbs). The commonly isolated bacteria from medical devices are *Staphylococcus aureus, Staphylococcus epidermidis,Pseudomonas aeruginosa, Haemophilus influenza, Pseudomonas aerobicus* and *Fusobacterium nucleatumand* among these *Staphylococcus spp* is more common.The Biofilm loaded devices can

affect virtually any organ or system of the human body and may cause infective endocarditis, cystic fibrosis, urinary tract infections, periodontitis, osteomyelitis and chronicwounds (Karygianni et al., 2020). Reports revealed approximately 65% of microbial infections of above clinical condition are related to biofilms. Resistance to antimicrobial agents and host defense systems is also enhanced by the attribute of biofilms. Thus biofilms create a considerable impact on human health and health care facilities.

Microbial Biofilms in Other Environment

Apart from food industry and medical field many other industries like paper manufacturing units, water treatment plants, drinking water channels, petroleum, nuclear power plant and marine industries are also affected by biofilms (Carniello et al., 2018). In fact, these industries are influenced directly and indirectly by biofilms that cause deterioration in machinery, equipment and quality of materials. Like presence of biofilms in water distributing pipes and channels lead to the contamination of water.

Tools to Combat Microbial Biofilm

Only because modern science has learned so much about the physiology of biofilms, it has now conceivable to develop efficient bacterial inhibition/dispersal strategies. Possible control strategies for bacterial biofilm may include preventing planktonic cell adhesion to surface and producing biofilm at first place or elimination of already formed biofilms (Van Holm et al., 2023). To limit microbial colonization on surfaces, the early attempt by bacterial planktonic cells to cling to surfaces must be inhibited before they organise into full fledge biofilm structure. This can be achieved either through surface treatment or by killing bacterial planktonic cells. Further, biofilm maturation can be avoided by controlling transcription of gene associated with the development of biofilm. Modern methods for removing biofilms often include antagonising QS signals, biofilm lattice inhibition, or killing the biofilm associated bacteria. Biofilms that have already developed can be eliminated by unsettling them and triggering their detachments.

Mechanical Disruption

Water-based sprays have been employed to mechanically disrupt biofilms, resulting in shear stresses. Ultrasound-induced biofilm dispersion is useful in the destruction of the bacterial biofilm when applied to solid metal surfaces like stainless steel.

Treatment with ultrasound changes the biofilm's shape and makes it more susceptible to antibiotics. Another method for reducing biofilm biomass by creating liquid shear pressures is laser-induced shockwaves (Burzell, 2022). Biofilms that have developed on biomedical apparatus can be disturbed by these shockwaves. Antibiotics are more likely to kill biofilms that remain following shockwave exposure. Another approach for passively disrupting biofilms is to apply a modest electrical current to the biofilm, which causes it to detach from the surface. The application of an electric current to electrolyze water molecules into hydrogen and oxygen gas bubbles at the corresponding electrode, enabling the biofilm to be disrupted.

Photodynamic Therapy (PDT)

The effectiveness of Photodynamic Therapy (PDT) against biofilms of Gram-negative and Gram-positive bacteria and fungi has been demonstrated in numerous investigations. Photosensitizing substances are used in PDT to activate singlet oxygen when exposed to light of a specified wavelength that the compound can absorb. A toxic-free dye and low-intensity visible light are used to create photosensitizing agents, which when combined with oxygen, form cytotoxic free oxygen radicals that induce photooxidation of many biological components (Hamblin & Hasan, 2004). There are many photosensitive agents, however only few of them are selected based on stringent criterion viz. should be non-poisonous, photostable and offer large quantum yield. Photosensitizers can be porphyrin derivatives (benzoporphyrins, trihydroxyanthraquinone, texaphyrin, phthalocyanines, naphthalocyanines, and protoporphyrin IX), tetrapyrroles derivatives (chlorins, bacteriochlorins and phthalocyanines, phthalocyanine) and phenothizine derivatives (Thioproperazine, Trifluoperazine Hydrochloride, Alimezine, Thioridazine Hydrochloride, Levomepromazine Hydrochloride, Promethazine Hydrochloride, Periciazine, Chlorpromazine Hydrochloride) (Oleinick et al., 2002).

PDT has become a popular alternative strategy for eliminating biofilms and offers a number of benefits over other methods. The actions of PDT include the rapid destruction of bacterial cells, reduction in biofilm thickness, and disintegration of the EPS structure (Dogsa et al., 2005). They work across a wide spectrum and are equally effective against drug resistant bacteria. PS-generated ROS have a short lifetime, and their efficiency decreases dramatically if the target is located far from the site of ROS formation due to diffusion hindrance. The yield of ROS is greatly influenced by the type of PS and hence creating great hindrance in achieving homogeneity affect. The efficiency of PDT in biofilms is also diminished by PS's inability to accumulate in biofilms or to penetrate to the bottom of EPS layers. The cationic PS display tenfold better effectiveness as compared to the anionic PS because they are trapped in the EPS matrix as a result of ionic or hydrophobic interaction (Ghorbani et al., 2018).

Photothermal Therapy (PTT)

A type of treatment known as photothermal therapy (PTT) employs the strong absorption of particular metallic nanoparticles and nanomaterials to locally heat a region. The hyperthermia generated by PTT compounds is largely employed to damage bacterial integrity or biofilm structure. Near infrared (NIR) wavelengths between 650 and 900 nm are the most effective for PTT, where it may penetrate the biofilm profoundly with little harm to the surrounding areas. PTT breaks down metabolic signals, denatures proteins and enzymes, and impairs membrane permeability to kill infections. PTT provides a number of benefits, including being effective, barely intrusive, and remotely controllable. In addition to having a broad antibacterial range, PTT does not result in bacterial mutations.

Photothermal agents are categorized as metal nanoparticles, carbon-based nanocomposites, and polymers. Metal nanostructures of various types, such as nanorods, nanostars, nanobipyramids, nanowires, and nanoworms (NWs), have been used as PTT agents. Among carbon-based nanocomposites, carbon nanotubes (CNs), and carbon quantum dots (CQDs) has been extensively studies. When combined with other treatments, such as photodynamic, PTT improves efficacy by providing synergistic antibacterial effects.

Microbial Enzymes

Anti-biofilm enzymes are regarded as novel and environmentally safe biofilm management agents, due to their ability to degrade extracellular matrix and promote biofilm dissociation. Bacterial biofilm extracellular matrix is consisting of nucleic acids, proteins, and polysaccharides hence, destruction of lattice of biofilm is possible by employing enzymatic lysis. A wide range of bacterial enzymes, for example proteases, glycosidases, and DNases, aid in the dispersal of active biofilms and increase cellular susceptibility to antimicrobials (Chew et al., 2019.). Formulations that contain enzymes capable of degrading microbial DNA, extracellular polysaccharides, proteinaceous components, and quorum-sensing molecules can more efficiently eliminate complex biofilms.

Exopolysaccharides are an essential element of bacterial biofilms and have a significant impact in growth and maintenance of the biofilm's integrity. Apart from being a nutrition binding matrix, it also helping with initial surface adhesion of bacterial cell, bacterial cell aggregation, water retention, mechanical stability, nutrient absorption, nutrient storage, enzyme binding, and functioning as a barrier against environmental stressors and antimicrobial chemicals derived from microorganisms. Therefore, glucosidase has broad applicability in managing biofilm infections by active polysaccharide breakdown. Glycosylated linkages between two or more carbohydrates are hydrolyzed by Dispersin B and other glycoside hydrolases (GHs).

Proteases produced by microbes control biofilms' dynamic architecture. This dynamic structure is essential for controlling biophysical processes associated with biofilm synthesis and maturation such as matrix remodelling and biofilm dispersal. One of the most efficient ways to disperse biofilms is by hydrolyzing the proteolytic adhesion of bacterial cells to solid surfaces, which also interferes with bacterial quorum sensing by disrupting signalling peptides. These proteases have a substantial effect on how biofilms are regulated in the organisms in which they are expressed and may also have an impact on biofilms from other species. Dispersal of bacterial biofilms is aided by metalloproteases and serine proteases produced by several bacterial species.

The enzyme oxidoreductase is produced by a number of wood-degrading fungal species, and it causes the oxidative degradation of glycans and oligosaccharides to produce the necessary lactones. These lactones hydrolyze on their own to form an unstable, ring-opened carboxylic acid. Reactive oxygen species (hydrogen peroxide) are formed as a result of this oligosaccharide oxidation process, and their accumulation has an antimicrobial effect. Extracellular DNA (eDNA), a vital and frequently sticky component, is also present in bacterial and fungal biofilms. EDNA-binding proteins, encourage the production of biofilms and crosslink the biofilm matrix to increase its stability by holding the bacterial extracellular DNA (Devaraj et al., 2019). Extracellular DNA's phosphodiester backbone is broken up into shorter sequences by the micrococcal nuclease enzymes, which reduces the DNA's sticky properties. Microbial lipase has a broad pH and temperature operating range, as well as high stability and activity. It has the ability to gradually hydrolyze triglycerides into glycerol and fatty acids and is recognised to be essential in the clearance of biofilm. Knowing that proteins, DNA, and polysaccharides are chief constituent of extracellular polymeric biofilm components, anti-biofilm enzyme mixture or anti-biofilm enzyme in combination with other substances may be a superior method of managing and eliminating biofilms (Bi et al., 2021). Used extensively in the biomedical, food, and healthcare industries, these cocktail enzyme formulations have already proven to be commercially viable (Table 18.1).

Phages

Phages are well adapted to break up biofilms since they are bacteria's natural enemies and can do so by entering the biofilm, disrupting the extracellular matrix, and infecting the bacteria. Phage therapy may work better and kill more biofilm bacteria if enzymes are used beforehand to dissolve the biofilm matrix. Additionally, the extracellular matrix is broken down by EPS-degrading enzymes produced by the host bacteria. The EPS breakdown aids phage penetration, growth, and pahge-mediated lysis of the bacterium. Lytic phages express the enzyme polysaccharide depolymerases. Polysaccharide depolymerases breakdown the polysaccharide framework and proteins in the biofilm (Srinivasan et al., 2021). Bacterial dispersion

Table 18.1 List of Microbial enzyme having antibiofilm activity

Class	Enzyme	Source	Target
Glycosidases hydrolases Hexosaminidases	Dispersin B	*Aggregatibacter actinomycetemcomitans*	Gram-positive & Gram-Negative bacteria biofilm
	Glycosidase pectinase	*K. oxytoca* af-G4	*Pseudomonas aeruginosa*
	Glycoside hydrolase Sph3h	Fungal origin	Activity against Pel and Pel-mediated biofilms
	Alginate lyase	Various algae	*Escherichia coli, Enterobacter aerogenes, Vibrio sp., Shigella flexneri*
	Pectinase	*Rhizopus sp., Siphoneugena densiflora (Myrtaceae)*	*S. aureus*
	Amyloglucosidase	*A. niger,*	*S. aureus* from polymicrobial biofilms
	Inulinase	*A. niger, Acinetobacter baumannii*	*Pseudomonas aeruginosa*
	Xylanase	*A. oryzae*	*P. aeruginosa* strains, PAO1
	A-amylase	*Bacillus strains*	*V. cholerae* and MRSA strains
	Cellulase	*A. niger*	*Burkholderia cepacia*Biofilms
Nucleases DNases	Dnase (nucb)	*Bacillus licheniformis*	Gram-negative and Gram-positive bacteria
Bacterial proteases proteases	Serine protease Esp	*Staphylococcus epidermidis*	*S. aureus* biofilm
	Neutrase	*B. amyloliquefaciens*	*S. aureus* and *S. epidermidis*
	Protease B	*B. licheniformis*	*Neisseria meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae*
	Subtilisin A(alkalase)	*B. licheniformis*	*S. marcescens* biofilms
	Metalloprotease serratopeptidase (SPEP)	*Serratia marcescens*	*P. aeruginosa* and *S. epidermidis*
	Subtilisin A	Bacillus genus	*Escherichia coli* staphylococcal biofilms
	Lasb elastase	*P. aeruginosa*	*Escherichia coli* staphylococcal biofilms
	Proteinase K	Engyodontium album	*Escherichia coli* staphylococcal biofilms

(continued)

Table 18.1 (continued)

Class	Enzyme	Source	Target
Endopeptidase	Bacteriolysin, lysostaphin	Staphylococci	Antibiotic-resistant *S. aureus strains*
Oxidoreductases	Cellobiose dehydrogenase	***Lignocellulolytic fungi***	Clinical S. epidermidis and Pseudomonas strains
	Hexose oxidase	Yeast Hansenula polymorpha	Staphylococcus aureus, methicillin-resistant S. aureus and Pseudomonas strains
	Glucose oxidase	Aspergillus species	Staphylococcus aureus, methicillin-resistant S. aureus and Pseudomonas strains

from biofilm is initiated as a result of localised bacterial lysis caused by a phage, as well as the accompanying enzymes degrading the bacterial cell wall and EPS.

The defence mechanisms of biofilms can prevent phage infection by modulating phage adsorption, entry, dispersion, and multiplication within biofilms. Factors such as biofilm framework organization, thickness of biofilm matrix, biofilm maturation stage, and the type of the constituent bacterial strains may limit phage infection and biofilm activity. In order to prevent phage infection, bacteria use the restriction-modification (R-M) mechanism, which involves specifically identifying and destroying phage nucleic acids Phages can penetrate the inner layers of a biofilm and can also reversibly bind to the bacterial adhesins in order to gain entry.

Monophages often have a limited host range since they are usually specific for a few strains of a bacterial species. Therefore, phage combinations, bioengineered phages, and phage-derived enzymes have all been employed to increase effectiveness and widen the spectrum (Maciejewska et al., 2018). The prerequisite for removing bacterial biofilms is the use of lytc bacteriophages that are incapable of lateral gene transfer of any virulence, toxin, or antibiotic resistance genes, and they should not be able to transduce infected bacterial cells.

By selecting phages with increased specificity, lysis capacity, reduced resistance, or avoiding lysogenic strains, the overall efficacy of phage therapy can be increased significantly. Phage application rate is critical since greater phage dosages result in a considerable decrease in phage output. Similarly, limited phage application may result in inadequate phage progress into biofilms.Genetic engineering has permitted the development of phages that encode peptidoglycan hydrolases that facilitate phage adsorption by unmasking receptors, penetration, and diffusion through the EPS-matrix for biofilm eradication (Clokie et al., 2009). Examples of phage-derived products that are easier to use than phages themselves are lysins and depolymerases. Phage cargoes can also be tailored to include nucleic acids, nanomaterials, pharmaceuticals, and diagnostic probes. The temperate phages might be employed as carriers for CRISPR-associated nuclease to reverse plasmid-mediated antibiotic resistance. Lytic phages along with their derivatives are typically used in tandem with antibiotics in combination treatment (Table 18.2).

Table 18.2 Advantage and Disadvantages of phage therapy

Advantages	Disadvantages
No toxicities or side effects	Narrow host ranges
Bactericidal rather than bacteriostatic in action	Anti-phage adaptive immune responses
Minimal impact on normal flora bacteria	Horizontal transmission of potential virulence factor
Effective against antibiotic resistant bacteria	Differences in pharmacokinetic behavior
Genetic modification is possible	Unknown safety or therapeutic efficacy
Less impact of phages on environments	Absence of regulatory framework for phage therapy
Relatively low concentration dosing	Challenging to identifying suitable phages

Antibiofilm agents (ABA)

Antibiofilm agents (ABA) are inorganic or organic chemicals that can inhibit or check the growth of microbial biofilms and broadly classified in two categories natural and synthetic.

Natural Antibiofilm Agents

Plant-Based Antibiofilm Agents

Antibiofilm agents derived from terrestrial and aquatic plants as well as microorganisms have been identified. Among the compounds on the list are phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and polyacetylenes (Bashir & Kumar, 2021). Numerous bioactive compounds with anti-biofilm activity have been identified from Indian medicinal plants, active against a variety of Gram positive and Gram negative organisms. These phytochemicals primarily disrupt the quorum sensing network by blocking quorum sensing inducers (Table 18.3).

Marine Natural Products

Marine flora and fauna are source of several natural materials, which have been tested for antibiofilm activity. Flustramine C analogues derived from the bryozoan Flustra foliacea inhibited Methicillin Resistant Staphylococcus aureus (MRSA), *A. baumannii*, and *E. coli* biofilm synthesis. Brominated guanidinium oxazolidinones, known as synoxazolidinones, were identified in arctic permafrost from *Synoicum pulmonaria* with strong action against Gram-negative acterial biofilm (Tadesse et al., 2010). Bufotenine, discovered in the Mediterranean coralline algae *Paramuricea clavate*, has been demonstrated to inhibit the adherence of the marine

Table 18.3 Natural Antibiofilm agents

Compound	Active ingredient	Source	Antibiofilm activity
Anthraquinone	Emodin	Roots and barks of numerous plants, molds and lichens	*P. aeruginosa and Stenotrophomonas maltophilia*
Flavonoids	Phloretin	Apples	*E. coli O157:H7 biofilm*
	Baicalin	Roots of Scutellaria baicalensis	*Burkholderia cenocepacia*
	Naringenin 2,	Citrus fruits	*V. harveyi and E. coli*
	Proanthocyanidins,	Cranberry plants	*P. aeruginosa*
	Stilbenoid resveratrol	Skin of grapes and berries	*Vibrio cholerae*
	Ajoenes	Extracts of garlic	*P. aeruginosa*
	Gingerols	Extracts of ginger	*P. aeruginosa strain* PA14
	Hyperforin	Hypericum perforatum St. John's Wort	*S. aureus* ATCC 29213,MRSA, *Enterococcus faecalis* ATCC 29212
	7-Epiclusianone	Rheedia brasiliensis	*Streptococcus mutans*
Triterpenoid	Isolimonic acid	Citrus plants	*Vibrio harveyi, E. coli* O157:H7
	Chelerythrine	Chelidonium majus	*S. aureus ATCC 6538P and S. epidermidis ATCC 35984*
	Casbane diterpene	Croton nepetaefolius	*Gram-positive* and *Gram-negative bacteria*
	Proanthocyanidin A2-phosphatidylcholine	Krameria lappacea	*Staphylococcus*
Polyphenolic compound	Tannic acid	Teas and other plant-derived foods	*S. aureus biofilm*
	Ginkgolic acid	Ginkgo biloba	*E. coli O157:H7*
	Quercetin	Fruits, vegetables and grains	*Streptococcus pneumoniae*
Essential oils	Carvacrol	Oregano	*S. aureus and S. epidermidis, S. typhimurium*
	Thymol	Oregano	*S. aureus, E. coli*
Plant alkaloid	Bgugaine	Arisarum vulgare	*P. aeruginosa*

bacterium *Pseudoalteromonas* spp. (Ponti et al., 2014). Ageloxime D, a diterpene alkaloid derived from the marine sponge *Agelas nakamurai*, blocks *S. epidermidis* from forming biolms (Choi et al., 2020). Darwinolide, a derivative of the Antarctic coral *Dendrilla membranosa*, inhibits MRSA biofilm formation. Bromoageliferin, derived from sea sponges, inhibited the production of biofilm by *P. aeruginosa,A. baumannii,S. aureus*, and *Bordetella bronchiseptica*. Meridianins are secondary chemicals produced from the sea mollusk *Aplidium meridianum* that

inhibit the growth of MRSA biofilms. *Delisea pulchra*, a marine macroalga rich in halogenated furanones, has been shown to interfere with quorum sensing by competing with LuxR-type receptors known to inhibit the development of *S. enterica* and *P. aeruginosa*.

Biosurfactants

Biosurfactants are a diverse category of amphiphilic chemicals generated mostly by microorganisms that aggregate at the interface between liquid phases, reducing surface and interfacial tension. They have acclaimed anti-adhesive, antibacterial, and biofilm disrupting abilities. Biosurfactants are preferred choice owing to highly selective action, selectivity, low cytotoxicity, great biocompatibility, high biodegradibility, and effectiveness at extreme pH and temperature (da Silva et al., 2021). Biosurfactants are frequently found in mixtures with isomers hence their purification labor-intensive or expensive. Tetrasodium EDTA and thiazolidione derivatives are the two most often used biosurfactant antibiofilm agents (tEDTA). Glycolipids are among the most researched categories of biosurfactants in other domains, despite the fact that they are underused as biofilm dispersion agents. N-acetylcysteine disrupts existing biofilms in order to exert their effects (Maier, 2003) (Table 18.4).

Antimicrobial Peptides

The natural antimicrobial/host defence peptides or small synthetic peptides are a separate class from antimicrobial peptides. Several new antibiofilm peptides have been identified that target numerous types of bacteria in biofilms, including significant clinically important antibiotic-resistant Gram-negative and Gram-positive bacteria. Many antimicrobial peptides have antibiofilm action in addition to their

Table 18.4 Biosurfactants used for antibiofilm activity

Compound	Active ingredient	Source	Antibiofilm activity
Lipopeptides	Fengycin-like lipopeptides	*B. subtilis and B. licheniformis*	*S. aureus and Escherichia coli*
	Putisolvin	*P. putida*	Pathogenic Pseudomonas sp. strains
	Pseudofactin	*P. fluorescens*	*Enterococcus faecalis*, *E. coli*, *Staphylococcus epidermidis*, *Enterococcus hirae* and *Proteus mirabilis*.
Cyclic peptide heptamer	Surfactin	*B. subtilis*	Salmonella sp.
Glycolipids	Rhamnolipids	*P. aeruginosa*	*Bordetella bronchiseptica*, *Bacillus pumilus*, *Candida tropicalis*
	Sophorolipids	*Candida sp.*	*Bacillus subtitlis*

effectiveness against planktonic bacteria. Antimicrobial peptides' antibiofilm actions include blocking bacterial cell adhesion at the start of the biofilm, decreasing biofilm maturation, removing already-formed biofilms, and/or dispersing the cells inside the biofilm. Additionally, antimicrobial peptides have the ability to disrupt the bacterial cell signalling system and degrade the extracellular polymeric matrix of bacterial biofilms(Huan et al., 2020). In addition to targeting a severe stress response in both Gram-negative and Gram-positive bacteria, antibiofilm peptides can also downregulate genes essential for biofilm formation and the movement of binding proteins(Fong & Yildiz, 2015). Based on the net charge they carry, AMPs can be categorised as either anionic or cationic AMPs. Interestingly, the great majority of bactericidal AMPs are cationic. These cationic AMPs attach to the anionic bacterial cell surface, causing bacterial cell lysis via membrane breakdown, impairment of cell wall synthesis, cell division, and suppression of LPS transport. The development of biofilms in bacteria is regulated by guanosine tetraphosphate (p) ppGpp, which is also involved in controlling growth and a number of other stress responses. When the AMPs reach the bacterial cell, they attach to the (p)ppGpp and cause it to degrade. Numerous peptides have been identified which disrupt the framework of biofilms by inhibiting matrix formation or promoting matrix breakdown. Most clinical strains of bacteria are typically sensitive to one class of AMPs or another, and resistance crossover to AMPs appears to be rare.

To avoid being destroyed by antimicrobial peptides, bacterial species have developed a variety of coping mechanisms. Gram-negative bacteria can release a number of compounds that can serve as a trap for antimicrobial peptides, such as alginate. The majority of the molecules that make up EPS have a negative charge, which may keep AMPs away from the biofilm by repelling them electrostatically through the positively charged peptides. The alternation of net-negative charge on the bacterial cell surface may interfere with electrostatic attraction to cationic AMPs. This can either be done by suppressing and/or changing the production of LPS or by functionalizing the part of lipid A by adding a phosphoethanolamine moiety. Esterification with a lysine residue and the addition of phosphatidylglycerol to teichoic acids can have a similar impact on Gram-positive bacteria (Brown et al., 2013). Increased resistance to antimicrobial peptides may result from modification of the phosphatidylglycerol (PG) group linked with the peptidoglycan sacculus in Gram-positive bacteria, which is mediated by bacterial membrane protein. AMP-EPS interaction may alter their antimicrobial effectiveness, posing a barrier to their development as antibiofilm medicines. A number of bacterial proteases have been discovered that can degrade AMPs. Several extracellular proteins of bacterial origin that can inactivate AMPs by binding to key metabolic enzymes have been identified (Bahar & Ren, 2013). Gram negative bacterial Outer membrane vesicles (OMV) are spherical bilayer structure produced in response to stress. These OMP can sequester free AMPs, before they can interact with bacterial cell. Also in many bacteria functional bacterial efflux mechanisms will efficiently flush out AMPs out of bacterial cells. By inhibiting their synthesis or increasing the production of host proteases that break down HDPs, certain bacteria can alter how HDPs are expressed in host cells.

Despite its numerous benefits, therapeutic use of antimicrobial peptides is fraught with complications. The protocol for synthesis and usage of AMP is still in its infancy, and optimization is required to realise its full potential. Host proteases' ability to break down AMP might prevent it from working properly. The AMP molecules have an innate tendency to form molecular aggregates, rendering them useless. The concentration of AMPs at the site of action is decreased by the spontaneous production of binding proteins by certain bacterial species. Antibiofilm peptides are presently only used to treat skin and soft tissue infections due to the fact that the safety profile of AMP therapy is still being studied (Table 18.5).

Metabolite Molecule

Marine species, particularly Alcyonacea and ahermatypic coral, sessile marine sponges, marine plants and macroalgae, produce secondary metabolite that has inhibitory effects on biofilm. Numerous metabolites from various marine species have been isolated, described, and shown to be excellent candidates for use as antibiofilm agents. In addition, the marine symbiotic bacteria are also known to produce some of the inhibitory compounds. These secondary metabolites have several important functions, one of which is to prevent the growth of biofilms by deactivating quorum sensing signals. Some of these metabolites have enzymatic activity, which aids in the degradation of biofilm-polymer by disrupting the signals. They interfere with the formation and integrity of biofilm and reducing the bacterial growth density.

Synthetic Antibiofilm Agent

Nanoparticles

Most antibacterial medications are rendered completely ineffective by the biofilm EPS matrix. The application of nanoparticles is one strategy to overcome this disadvantage by helping to penetrate biofilm armour. The majority of these nanoparticles are formed of inorganic materials, such as metal oxide nanoparticles; however, due to the flexibility of their design, organic nanoparticles are popular choice as delivery systems for antibiotics with sustained drug release. These nanocarriers successfully capture medicinal molecules, preserving them from biodegradation and increasing their efficiency. As biofilm-targeting agents, nanosystems with intrinsic antibacterial activity can be utilised. Due to their substantial total surface areas and direct interaction with microorganisms, nanoparticles exhibit effective antibacterial activity. Following nanoparticle attachment, the bacterial cytoplasmic membrane is pierced and the nanoparticles kills bacteria by interfering with protein synthesis mechanism either by DNA damage, disrupting process of translation, and/or upsetting transcription after penetrating. Utilizing nanoparticle therapy in conjunction

Table 18.5 List of bioactive antimicrobial peptides

Peptide	Source	Target	Action
Protegrin 1	Leukocytes	Board spectrum of pathogens including multi-drug resistance bacteria	Membrane disruption by forming a pore/channel
Pleurocidin	Skin mucous secretions	Gram-negative bacteria	Bacterial cell membrane damage
Piscidin 3	Fish peptide	Gram-positive and Gram-negative bacterial pathogens	Degradation of extracellular DNA
Indolicidin	Bovine neutrophils	Gram-positive and Gram-negative bacteria as well as fungi	Inhibits DNA synthesis
SMAP-29	Sheep leukocytes	Burkholderia thailandensis	Pore formation on bacterial cell membranes
β defensin 3	Skin, tonsils, oral/saliva,	Gram-positive and Gram-negative bacteria as well as fungi	Reduce the expression of polysaccharide intracellular adhesin (PIA)
Nisin A	Lactococcus and Streptococcus species	Gram-positive bacteria and is particularly effective against bacterial spores	Degrade the membrane of biofilm-embedded cells
Cathelicidin LL-37	Secondary granules of neutrophils	P. aeruginosa biofilm	Affect the bacterial cell signaling system
Hepcidin 20	Liver	Wide range of fungi, bacteria and viruses	Inhibits iron transport by binding to the iron export channel ferroportin
Cecropin A	Haemolymph of giant silkworms, *M. domestica*, arge roundworm *Ascaris suum*	Gram-positive and Gram-negative bacteria as well as fungi	Pore-formation
Melittin A	Honeybee *Apis mellifera*	Methicillin-resistant Staphylococcus aureus (MRSA) biofilm	Pore-formation
Pyrrhocoricin	*Pyrrhocoris apterus* (Sap sucking bug)	Gram-negative bacteria	Prevention of protein folding aided by chaperones
Temporin L	Skin secretions of the European red frog *Rana Temporaria*	Gram-positive and Gram-negative bacteria	Destabilize microbial cytoplasmic membrane
Buforin II	Stomach tissue of the Asiatic toad *Bufo bufo gargarizans*	Gram negative bacteria	Inhibits the cellular functions by binding to DNA and RNA of cells
Protegrin-1	Porcine leukocytes	Gram-positive and gram-negative bacteria	Induces membrane disruption by forming a pore/channel

with external stimuli including pH, light, and magnetic fields can enhance the antibiofilm action.

Following nanoparticle buildup in the biofilm area, it adheres to the external surface of biofilm matrix and migrates milieu interieur. The physicochemical features of the EPS, the milieu surrounding the biofilm, and the zeta potential of the nanoparticles all have a significant impact on the EPS-nanoparticle interaction. Electrostatic attraction allows a negatively charged matrix to easily interact with cationic nanoparticles. Nanoparticles are distributed and diffuse into the biofilm after entering the matrix of extracellular polymeric substances (EPSs). The dispersal of nanoparticles inside the biofilm is influenced by matrix pore size, the existence of aqueous pores, ambient lipophilicity, and the polarity of the EPS and nanoparticles. Ion concentrations differ in the aqueous channels of biofilms. The ion composition and concentration determine nanoparticle penetration into the biofilm.

The specific advantages of nanosystems includes have high drug encapsulation efficiency, release of drug over extended period of time, improved stability, better drug bioavailability, and greater accumulation at biofilm scaffold. Bioengineered nanoparticles can be designed in such a manner that activation by different stimuli causes the photothermal or photodynamic erosion of biofilm matrix. Bacterial colonisation in biofilms has been effectively prevented or treated using nanoparticles as nanocarriers for antibiofilm agents. Due to the presence of antibacterial components such as molecular oxides and macrocyclic surfactants, certain nanoparticles can also have antibiofilm properties on their own. There are several types of nanoparticles used with the intention of biofilms destruction.

Inorganic Metal-Based Nanoparticles

Metals and metal oxides are examples of rigid particles comprised of diverse materials. Some inorganic nanoparticles can interact with EPS due to charged functional groups present on surface or electrostatic interactions, while others can significantly alter the microbial EPS through targeted release of ions. Inorganic nanoparticles exerts its antibacterial mechanisms through mechanical membrane damage caused by electrostatic contact, oxidative stress caused by ROS formation, lipid peroxidation, mechanical destruction of the EPS architecture, and interference with protein function caused by metal ion release.

Organic Polymer Nanoparticles

Several organic compounds like lactic acid, glycolic acid, caprolactone, ethyleneimine, acrylic acid, glutamic acid, and cellulose have been used to make polymeric nanoparticles scaffolds for therapeutic or drug delivery purposes. Multiple medications may be contained by polymeric nanocarriers, which makes synergic treatment possible. The nanostructure should either be physically loaded with these

medications or covalently coupled to them. Polymeric nanocarriers containing antibiotics are widely used to treat biofilm infections. It has been shown that polymeric nanosystems naturally inhibit biofilm growth. Because the outer layers of EPS have negative charges, these nanoparticles function through electrostatic interactions. Polymeric nanoparticles are distinguished by their controlled qualities matched to a specific payload and to the suitable size, as well as their ease of functionalization. Polymers have minimal toxicity and good biocompatibility when it comes to medication delivery.

Lipid-Based Nanoparticles

Lipid-based nanoparticles contain lipid-rich nanosystems either on the surface or in the core matrix of the particle. Based on the nature of the internal matrix, they are classed as solid lipid nanoparticles (SLNs) or nanostructured lipid carriers (NLCs). Solid lipid nanoparticles (SLNs) are nanocarriers with solid lipid cores in the 10–1000 nm range that can hold medicinal active substances that are both hydrophobic and hydrophobic at room and body temperatures. Solid and liquid lipids that are compatible and biodegradable, as well as hydrophilic emulsifiers, make up nanostructured lipid carriers, or NLCs.

Conclusion

The biofilm-forming ability of bacteria is viewed as a serious concern in several domains related to the food and healthcare industries. Bacterial biofilm has the ability to contribute to disease pathogenesis in numerous possible ways, either by increasing bacterial resistance to the body's defensive mechanisms or by making them resistant to antibiotic therapy. Improved understanding of the molecular process of biofilm formation has resulted in the development of novel biofilm remediation technologies and the identification of several potential biofilm removal agents, which have significantly aided in the medical management of biofilm-related complications. The comprehensive characterization of extracellular polymeric compounds will be more precisely conducted by employing optical imaging techniques like confocal microscopy. Nanobots and nanorobotics in conjunction with MALDI-MS are promising future areas to unearth the mysteries of complex biofilm microenvironments. In order to monitor variability in the biofilm milieu, the majority of studies investigating biofilm mechanisms use transcriptomics and proteomics approaches, which involve the use of enormous and complex data sets. Machine learning and artificial intelligence will be more widely used to scale up data analysis with much higher accuracy and speed.

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Chapter 19
The Role of Air and Aerosols in Contaminating Food Products During Food Processing

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Introduction

The presence of airborne organisms is a significant challenge confronting the food industry. Airborne organisms are typically generated through droplets deposited in aerosols with diameters ranging from 0.5 to 50 μm (Brandl et al., 2014; Lee, 2011; Stetzenbach et al., 2004). Aerosols are microscopic particles in the air in the form of liquid or solid particles (Sutton, 2004). These aerosols can carry bacteria, mold spores, yeasts (Brandl et al., 2014; Lee, 2011; Sutton, 2004), and pathogenic microbes, that then become bioaerosols. Pathogens that could be found in food-processing bioaerosols include *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus*, and *Clostridium* spp. (Masotti et al., 2019a, b). Hence, airborne organisms are considered one of the contributing factors to the cross-contamination of food and food contact surfaces (Masotti et al., 2019a, b). The food industry, especially food processing areas, is subject to many processing activities, including spraying, splashing, and employee movements. Those activities always create aerosolization and droplets that can persist in the plant's environment (Sutton, 2004; Xie et al., 2021). The droplets from aerosolization may hold airborne organisms and become a source of contamination (Masotti et al., 2019a, b). Airborne microorganisms in the form of bioaerosols can readily spread through the air and consequently contaminate food, and food contact surfaces, such as processing

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equipment, containers, conveyors, and other equipment (Brandl et al., 2014; Otto et al., 2011). Bioaerosols can also transmit from food processing to other areas such as chilling, packaging, and dry storage areas, in food establishments through the employees' movement or tools and equipment, leading to negative effects on the quality of food products and potential foodborne infections (Madsen et al., 2020; Mohammad et al., 2021). Usually, the processing environment is moist due to the processing activities mentioned above. Therefore, various factors affect the movement and direction of the air in this critical area, which also affects airborne microorganisms (Masotti et al., 2019a, b). Typically, food products remain longer in the processing area before being transferred to other sites and being exposed to air or aerosols for an extended period of time (Sutton, 2004). Thus, air monitoring and preventive programs are required to avoid potential cross-contamination because appropriate airborne control measures ensure the safety and quality of food products (Masotti, Cattaneo, et al., 2019a). However, it has been found that the concentration of airborne microbes varies within food establishments, with the average level being low (Mohammad et al., 2021; Okraszewska-Lasica et al., 2014; Pearce et al., 2006; Sutton, 2004).

Airborne microorganisms are expected to always be present in food processing establishments, such as poultry, beef, dairy, and pork, because animals are reservoirs of bacterial pathogens, such as *Salmonella*, *E. coli*, and others. As a result, food industries must evaluate the bioaerosol levels in their facility's environment and assess the quality and shelf life of food products. Evaluating and identifying the potential airborne sources and types of microorganisms in food processing establishments is critical for developing effective preventive and hygiene practices and reducing microbial risks. This can be achieved by air sampling the entire food plant and evaluating the microbial load (Moracanin et al., 2019; Napoli et al., 2012). Many studies assessed the level of bioaerosols in the air of food processing plants and obtained beneficial results (Altunatmaz et al., 2012; Duggan et al., 2010; Pathak & Verma, 2013; Mohammad et al., 2021; Okraszewska-Lasica et al., 2014; Pearce et al., 2006; Sutton, 2004; Wu et al., 2018). The results from studies on beef, pork, and livestock establishments confirmed the presence of airborne pathogens at processing sites. For example, Okraszewska-Lasica et al. (2014) evaluated three commercial beef, sheep, and pig plants for the presence of *Salmonella* spp. and *L. monocytogenes*. The authors reported that both pathogens were detected in all three facilities. However, they confirmed that *Salmonella* levels were higher in the pig plant, while *L. monocytogenes* were mainly found in the beef plant. Mohammad et al. (2021) collected air samples from two small and two large commercial beef abattoirs to evaluate the presence of *Salmonella* and *E. coli*, comparing two different detection methods. The authors found both *Salmonella* and *E. coli* in all plants by both methods. However, they reported that the prevalence of both pathogens was affected by the facility size and the processing stage. Another study by Pearce et al. (2006) examined the prevalence and distribution of airborne *E. coli* and *Salmonella* in a pork slaughtering establishment, and the study revealed that both pathogens were detected at different stages and levels. Therefore, the food industry should pay additional attention to their hygiene practices and keep the food processing

environment clean. Food processing management needs to monitor the flow of air direction and ensure their ventilation system is effective and working (Beck et al., 2019; Wray, 2011). To this end, novel and innovative emerging technologies would be a great addition to overcoming the challenges and risks of airborne contamination in the food processing environment. Hence, this chapter covers the transmission and sources of airborne organisms in the food processing environment, identification and detection methods, and control of airborne in food processing establishment, novel, and emerging technology to prevent, control, and inactivate airborne organisms, and factors affecting their presence and concentrations.

Source and Transmission of Airborne Microorganisms in the Food Industry Environment

The source of airborne microorganisms in food processing environments varies and can be from different sources, depending on the size, activities, and sanitation practices of food plants. Airborne microorganisms are typically in the form of droplets known as bioaerosols (Sutton, 2004). Bioaerosols can be produced by wastewater, rinse water, aerosolized spilled products, air-conditioning systems, food production systems, raw ingredients, and worker activity (talking, sneezing, and coughing) (Mohammad et al., 2021; Nerin et al., 2016; Sutton, 2004). Heldman (1974) found a strong correlation between worker activity and airborne bacteria. Bioaerosols can also be generated through operation equipment, sink, floor drain, and high-pressure spraying (Mohammad et al., 2021; Sutton, 2004). Food processing environments typically have high moisture, ventilation and air conditioning systems, and heating, which provide an ideal environment for the growth of microorganisms and the consequences of airborne microbe growth (Altunatmaz et al., 2012; Nerin et al., 2016). Therefore, air sampling of food in the processing environment helps identify the sources of airborne microorganisms and potential contamination with airborne pathogens (Gollakota et al., 2021). The food industry can also use air sampling to determine the risk of airborne contamination and prevent the further spread of airborne microorganisms (Gollakota et al., 2021; Napoli et al., 2012).

Airborne or bioaerosols usually spread to food and food contact surfaces through the air. Therefore, many factors contribute to the transmission of airborne organisms from the air to food, food contact surfaces, and processing equipment (Brandl et al., 2014). The most common factors contributing to the spread of bioaerosols in food processing environments include the construction of food plants (doors, drains, etc.), activities during cleaning processes and disinfectant, washing, packing, incorrectly or inadequately designed and mainlined ventilation and air conditioning systems, and of course, employees and people activities, and poorly constructed interior and roof structures that lead to drainage or leakage (Moracanin et al., 2019). Hence, in the food processing environment, some of the above-mentioned factors usually allow microorganisms suspended on particles in bioaerosols to transmit to the food

products or food contact surfaces, resulting in contamination. Additionally, many other factors such as temperature, humidity, airflow, and nutrient sources, provide conditions for the growth and transmission of airborne organisms (Moracanin et al., 2019). Thus, air is not ideal for airborne growth if moisture, nutrients, and the correct temperature are unavailable in the plant's environment (Moracanin et al., 2019; Sutton, 2004). However, any point where food products or food contact surfaces are exposed to air is considered a route for airborne transmission. Air serves as a transient place but not as a source of airborne microbes. In this case, if airborne microorganisms are present in food processing environments, there is a suitable condition for their growth and survival and potential contamination risks are present (Moracanin et al., 2019). Therefore, the food industry should pay additional attention to their plant environment, identify potential sources of airborne transmission, and avoid any sources that lead to the generation of bioaerosols. Airborne microorganisms may persist in aerosols derived from activities, such as water spraying and sanitation in food processing establishments, and multiply, which may lead to food contamination. Identifying the sources of bioaerosols and the transmission of airborne microorganisms is of utmost importance for understanding the role of air in the food processing atmosphere and controlling the spread of potential contaminants.

Identification and Detection Methods of Airborne Contamination in Food Processing Environments

The transmission of airborne microorganisms is a food industry concern because they can contaminate foods and food contact surfaces and cause foodborne diseases. Therefore, effective monitoring procedures and robust and timely detection methods are essential to control and prevent airborne contaminations and potential foodborne illnesses from pathogenic microbial particles in aerosols (West & Kimber, 2015). In the food industry, monitoring air quality and microbial concentration is implemented by collecting air samples and identifying the microbial load through proper quantitative or qualitative analyses of collected bioaerosols. However, the results are significantly affected by the air samples collection, the type of air sampler, the sample size selected for analysis, the collection medium (which may affect the level of microbial recovery and viability), and the detection methods, quantitative versus qualitative (Dybwad et al., 2014; Hoisington et al., 2014). Therefore, an adequate air sampler for aerosol collection, isolation of airborne microorganisms, the concentration of the samples, and differentiation and detection methods of pathogens should be the focus to ensure effective detection and identification. Typically, air samples are collected using two methods (passive and active) (Okraszewska-Lasica et al., 2014). Different air samplers and their advantages and disadvantages are shown in Table 19.1.

Table 19.1 Methods of collecting airborne microorganisms

Method	Advantages	Disadvantages
Settle plates (sedimentation)	Reliable Easy Cheap No stress to microorganisms Standard	Low correlation with counts Inability to measure the number of viable particles per volume of air Long sampling times Bias to large particles Low correlation with counts
Impingers	Easy to implement Cheap Good for highly contaminated environments	Liquid impingers used for areas high concentration bioaerosols Cannot collect bioaerosols particles smaller than 1 μm
Impactors Slit	High recovery rates Low sampling stress, No additional steps are needed after air collection High sampling efficiencies Simple to operate	Complex and huge to handle Expensive, unmanageable Unsuitable for large outdoor air collection Long-time sampling
Impactors Sieve	Multiple flow rates Small size allowing for easy placement Virtually no particle generation Comparable recover to traditional Slit-to-Agar designs Standard 90 mm test plates	Complex and huge to handle, expensive
Cyclonic separation	Ability to collect large volumes of air continuously for a long time Collect bioaerosols into a liquid solution Airflow rates of 100–300 L/ min High effective A wet collection system protects cells from osmotic stress	Selective for large air particles Higher counts than other air samplers
Electrostatic precipitators	High particle collection efficiency High sampling rate Less resistance to airflow.	Produce ozone and nitrogen oxide, Subject microorganisms to toxicity Complex and requires professional management and handling.
Thermal precipitation	Adequate for collecting particles smaller than 5 μm Helpful for microscopic investigations	Not typically used in the food industry Requires accurate adjustments collects low-rate air sampling ranging from 300 to 400 ml/ min.

(continued)

Table 19.1 (continued)

Method	Advantages	Disadvantages
Centrifugation samplers	Subject microorganisms to less stress Does not create high-velocity provide more representative samples High air volume Simple Cheaper than impactor methods.	Only suitable for big particles
Filtration	Easy Fast Flexible Cheap Used to quantify mold and bacteria Collect large volumes of air short sampling time	Exposed cells to stress

Settle Plates In passive methods, aerosols are collected using settle plates (Petri dishes) by exposing the open plates containing non-selective medium to the air for a specific time and incubating overnight, then counting colonies (Dybwad et al., 2014; Sutton, 2004). This sample collection method is limited as the plates only collect viable airborne microbes that are sediment from the air and settle onto the agar surface within the exposure time. It is only suitable for bigger aerosol particles and cannot detect small ones. Additionally, with settle plates, it is not possible to collect a specific amount of air; therefore, the results could be more qualitative (Sutton, 2004). The settle plates become overgrown in a facility with high airborne concentrations, resulting in uncountable colonies (Hoisington et al., 2014). The settle plates can also become contaminated with non-air particles and deteriorate and dry quickly, making it difficult to interpret results. Some advantages are that it is easy to use and can collect bioaerosols in their actual state. The main drawback of this method is its incapability to measure the number of viable particles per volume of air. Other drawbacks of using this method are sampling times as it takes too long, significant dependence on air currents, bias towards big particles, and low correlation with counts obtained using different methods. This approach is proper when falling out onto a specific area to determine the presence of airborne organisms.

In the active sampling method, usually, air samplers are used to collect bioaerosols, and different devices with different structures and functions are utilized, such as impingement, impaction, cyclonic separation, filtration, thermal or electrostatic precipitation (Masotti et al., 2019a, b). However, each of these devices gives different results for the same sampling site and at the same time due to their structure and properties differences (Masotti et al., 2019a, b; Verreault et al., 2011). In active air sampling, a determinate volume of air/aerosols can be collected from the food establishment testing sites using one of the above-mentioned devices (West &

Kimber, 2015). The active air samplers based on popularity for use in air sample collection are hereby described.

Impingers Impingers are air samplers that collect aerosols using a liquid medium. Typically, the airflow through the inlet facilitates air transfer to the liquid medium. When the air hits the surface of the medium, the suspended particles impinge on the collection liquid medium. An appropriate liquid medium such as peptone water, phosphate buffer saline, or nutrient buffer, must be used to ensure the recovery of various types of microorganisms, maintain the microorganism's viability, and at the same time inhibit their growth (Sutton, 2004). After sample collection, the total air and medium liquid volumes are determined. The collected air samples in a liquid medium are analyzed using culture or rapid detection methods (Sutton, 2004). The advantages of using impingers are that they are easy to implement and inexpensive. However, some limitations of liquid impingers include the fact that they are usually used in areas with high bioaerosol contaminations and that they cannot collect bioaerosol particles smaller than 1 μm (Sutton, 2004).

Impactors These types of air samplers are used by most of the food industry for bioaerosol collection. Impactor air samplers collect samples using a solid medium. The impactor employs a solid agar plate and has two stages of work based on the size differentiation of aerosol particles. After the air is sieved through a plate for particle collection, the air is directed by a vacuum toward the agar or adhesive-coated surfaces. Following sample collection, the plates are incubated for 24–48 hours, and the colonies are counted to determine the level of airborne microbes in the air. Two types of impactors are available: slit and sieve air samplers, which are different in their shape and functions (Sutton, 2004).

A slit air sampler usually comes in a cylindrical shape with a tapered slit tube and functions by pulling the air by vacuum; it has a tapered slit tube that forms a jet stream during air sample collection. The vacuum in a slit sampler requires a constant flow rate of 28.3 liters per minute (L/min) (Masotti et al., 2019a, b). This type of air sampler collects air onto an agar plate while the plate rotates, which allows for even particle distribution over the agar plates. An example of a slit air sample is STA, New Brunswick Sci. Co. Inc., Casella, BGI Inc.

Sieve impaction air samplers are a second type of impaction device. These devices function by using the acceleration of air with a rated flow of 28.3 L/min that moves particles through a sieve mesh (a metal plate with numerous small holes) (Sutton, 2004). The particles in the air then impact onto the surface of the agar medium. This is an aggressive and effective method of gathering air samples. Sieve air samplers comprise of single or multiple stages. For example, it may include one, two, six, or eight stages. The air samplers with multiple stages have smaller holes that increase based on each stage resulting in increased particle velocity while the air moves through the sampler. For example, large air particles are impacted in the first stages, and smaller particles stay to be impacted in the subsequent stages.

Typically, single-stage air samplers do not distinguish between particle sizes. Therefore, these types of impactors are used when the purpose is to collect the total

number of viable particles per unit of air volume (Sutton, 2004). The two-stage air impactors are used when the purpose is to discriminate between respirable and non-respirable particles and usually separate almost all the viable particles that are 0.8–5 μm in size (West & Kimber, 2015). Multiple-stage impactors are used when the purpose is to collect and enumerate viable particles per unit of air volume based on the size of particles in the bioaerosol (Xu et al., 2011). They are usually utilized in healthcare operations and are uncommon for food processing establishments. Impaction air sampling methods are widely used due to their higher recovery rates than other air sampling methods, especially in environments with low bioaerosol levels. Additionally, this method has low sampling stress, and no additional steps are needed after air collection as particles are on agar plates, possess high sampling efficiencies, and are simple to operate (West & Kimber, 2015). However, like any other device, sieve impactors have some drawbacks, including being complex and huge to handle, expensive, and unmanageable. Also, they require special care to maintain sterility inside the samplers and the agar plates outside to prevent contamination (Sutton, 2004). In addition, they are unsuitable for expanded outdoor air collection because these air samplers are designed to collect low flow rates of 1.5 to 300 L/min and require long-time sampling (Sutton, 2004).

Cyclone This type of air sampler collects aerosol particles into a liquid medium, unlike impactor air samplers that collect aerosol particles onto solid/semi-solid mediums, exposing cells to stress by filters trapping bioaerosol particles surfaces of fine fibers or porous membrane. On the other hand, impingers function by channeling air flow through nozzles into a chamber of liquid (Sutton, 2004). Bioaerosols are collected by cyclone-air samplers via the collection chamber into a spiral, swirling flow where they are exposed to a centrifugal force based on their diameter, density, and speed. The centrifugal force then separates bioaerosol particles from the air by transporting particles into a liquid with sufficient inertia toward the cyclone wall (Sung et al., 2017).

Compared to other air samplers, the cyclone has many advantages, including the ability to collect large volumes of air continuously and for a long time, collecting bioaerosols into a liquid solution with airflow rates of 100–300 L/min, which prevent cell stress due to sample drying. Cyclones are found to be highly effective at collecting bioaerosols (Sung et al., 2017). They can be used in real-time monitoring of microorganisms in the air (Sung et al., 2017), including pilot studies at poultry facilities. Particles in a large volume of air are concentrated in a relatively small amount of liquid and tested by qualitative or quantitative methods to determine bacterial pathogens' presence and/or concentration. A wet collection system protects cells from osmotic stress. An example of this type of air sampling is a dry, wet-walled, and two-stage cyclone.

Filtration This method of air sampling is based on the separation of aerosol particles from the air by passing the air through a filter for a certain time at the same speed (Sutton, 2004). Usually, the filter is attached to a holder and connected to a vacuum origin with a control flow rate (Sutton, 2004). The filter can consist of any

material, such as sodium alginate, cellulose fiber, glass fiber, gelatin membrane with a pore size of 3 μm, and a synthetic membrane with a pore size of 0.45 μm or 0.22 μm (Sutton, 2004). For direct culture-based analysis, gelatin membrane filters are used because they are water-soluble and are placed directly onto an agar surface for analysis and enumeration of microorganisms (Zand et al., 2022). In comparison, synthetic membrane filters are restless into a liquid before analysis. These air sampling methods are widely used because they are easy, fast, flexible, cheap, can be used to quantify mold and bacteria, and can collect large volumes of air within a short sampling time (Zand et al., 2022). However, they exposed cells to stress due to drying, making them less effective for most vegetative cells than other air samplers (Sutton, 2004). Example of this type of air sampling is Button, IOM, and virtual impaction (MVI).

Centrifugation Samplers Similar to cyclone air samplers, centrifugation air sampling approaches create a force that pushes airborne particles onto the surface of agar. Aerosol is moved in a circular motion at a high velocity, and the centrifugal force causes the particles to impact against an agar surface. The advantages of using these air sampling methods include less stress on microorganisms than other methods, such as impaction or impingement sampling methods, because centrifugation does not generate high-velocity jet forces during sample collection (Masotti et al., 2019a, b). Additionally, they can provide more representative samples because they are fast and collect a high air volume. They are also simpler and less expensive than impactor methods. However, centrifugation methods are only suitable for big particles. An example of these air samplers is the Reuter centrifugal air sampler (RCS Sampler, Biotests Diagnostics Co.), which is portable, battery-operated, easy to use, and can collect 100% of 15 μm particles and 55% to 75% of 4 μm to 6 μm particles (Oliveira et al., 2020).

Electrostatic Precipitators This air sample collection method consists of a glass chamber.

The air is drawn inside the chamber, and bioaerosols are subject to an electrostatic charge, and the charged air particles are then attracted to oppositely charged plates. Briefly, the air is drawn into a chamber, which is comprised of top and bottom parts (Masotti et al., 2019a, b). Inside the chamber, the air is subjected to an electrostatic charge of 13 kV. This high charge creates ions and ionizes the air and bioaerosols inside the chamber. After aerosols and droplets become charged, they are attracted by the oppositely charged plates at the bottom of the chamber, which is covered with collected medium. The advantages of using this method are high particle collection efficiency, high sampling rate, and less resistance to airflow. However, this method may produce ozone and nitrogen oxide, subjecting microorganisms to toxicity and death before enumeration. Additionally, this method is complex and requires professional management and handling (Masotti et al., 2019a, b; Oliveira et al., 2020).

Thermal Precipitation This air sampling method captures airborne microorganisms through the precipitation of bioaerosols using thermal precipitators through a temperature gradient. Like electrostatic precipitation, thermal precipitation also collects aerosols by drawing air onto a cylindrical chamber by suspending a wire that goes through the chamber with an airflow rate of 7–20 ml/min. The suspending bioaerosol particles then move from the high-temperature zone to the low-temperature zone. The thermal force here is only effective for a 750 °C cm^{-1} temperature gradient and less. This method is adequate for collecting particles smaller than 5 μm and helpful in microscopic investigations. However, thermal precipitation is not typically utilized in the food industry as it requires accurate adjustments and collects low-rate air sampling ranging from 300 to 400 ml/min (Masotti et al., 2019a, b; Oliveira et al., 2020).

To this end, the food industry commonly used settle plates or liquid impactor air samplers. However, wet-walled cyclones and liquid impingers with swirling liquid and the CIP 10-M provide additional advantages of keeping the viability of the cells and more accurate counts of airborne microorganisms compared to solid-based surfaces air sampling (Oliveira et al., 2020).

Analysis of Airborne Microorganisms After air sample collection, the air sample is analyzed to determine the concentration of airborne microorganisms using culture, rapid methods, or microscopic analysis (Mbareche et al., 2018; Reponen et al., 2011). Culture-based analysis is the most commonly used method in the food industry for counting airborne microbes as a direct method of analysis (Oppliger, 2014). However, selecting the appropriate air sample analysis technique is critical because not all air sampling systems used to collect air samples are compatible with specific analyses. For example, settled plates rely on culture-based analysis, while impaction air sampling methods use both culture-based and microscopy analysis. Impingement, cyclone, and filtration sampling methods are more suitable for analysis with rapid air samples, such as molecular or immunological approaches, as these methods collect air samples in the liquid or on a filter (West & Kimber, 2015).

Airborne microorganisms exposed to stress during sampling may not be able to grow under the nutrient medium used for culturing (Masotti et al., 2019a, b). In this case, using different types of nutrient media during sampling could help to recover the most microorganisms during the analysis. Nonselective agar, such as tryptic soy and nutrient agar, is usually used for culture-based analysis. After incubation, the culturable microorganisms are grown on the agar, and the airborne microorganism's concentration is determined by counting the number of colonies which are recorded as colony-forming units (CFU) (Napoli et al., 2012). Further analyses are performed for bacterial identification using biochemical tests, microscopic morphology, and Gram stain reactions to determine the types of airborne microorganisms. Direct culture-based methods are easy to use, reliable, and considered a gold standard. However, they are labor and material-intensive, inaccurate, time-consuming, unsuitable for nonculturable organisms, and subject to contamination (Vasavada et al., 2020).

Rapid analysis approaches like molecular and immunological approaches overcome these limitations of traditional direct methods. Immunological methods like enzyme-linked immunosorbent assays (ELISA), precipitin assays (immunodiffusion), and particle agglutination assays (latex agglutination) function based on the interactions between microbial antigenic and antibody (antigen-antibody) (Vasavada et al., 2020), whereas molecular approaches, like polymerase chain reaction (PCR), are based on the amplification of DNA or RNA analysis or amplification of the 16 S rDNA, then sequencing and DNA hybridization (Vasavada et al., 2020). The latter increases the sensitivity and specificity of the test and decreases the analysis time (Stetzenbach et al., 2004). To conclude, the food industry should consider both direct culture methods and modern rapid approaches for identifying the airborne concentration in their plants' environment.

Current and Emerging Technology to Prevent, Control, and Inactivate Airborne Contamination in Food Processing

The presence of airborne microorganisms in the air of food industry environments is random, and their load is variable, usually ranging from 10 to 10,000 CFU/m3 (Ehavald et al., 2007). However, understanding the level of microbial load and having information about bioaerosols is vital for evaluating the risk to food product safety and quality and protecting public health. Usually, air entering a food processing establishment from the outside is filtered and chilled to eliminate unwanted microorganisms that are expected to enter the plant's environment from the outside. However, factors such as processing activities, personnel, and facility structures cannot be fully controlled. They may contribute to the generation of droplets and bioaerosols that hold pathogenic microorganisms inside the food processing plant, which is variable among food processing plants and in the same facility based on the type of daily activities (Masotti et al., 2019a, b). Monitoring and evaluating airborne microorganisms using adequate air sampling and reliable and sensitive analysis techniques is the first step to preventing and reducing the occurrence of airborne contamination. The food industry is aware that monitoring airborne microorganisms has become a must, and now it is part of their quality control practices (Masotti et al., 2019a, b). Airborne microbial monitoring can be included as a section in the food industry HACCP plan (Beletsiotis et al., 2011; Oliveira et al., 2020).

Air disinfection methods are performed to reduce airborne microbial loads in the air of food plants in addition to their standard chemical sanitation practice. Air disinfection is implemented using chemical fogging, ozone treatment, UV irradiation, hydrogen peroxide, and cold plasma methods (Brown & Wray, 2014). Personal hygiene, preventing cross-contamination, zone separation, and water purification also assists in reducing airborne microorganisms (Gurnari & Gurnari, 2015). Proper food storage conditions, facility maintenance, and air filtration are effective ways to

improve food safety. Effective air quality management can significantly reduce airborne microorganisms in food processing environments. Proper ventilation removes moisture discharged during processing activities and prevents surface condensation and mold growth. Airflow is one of the significant factors contributing to the transmission of bioaerosols from dirty areas to clean areas in food processing facilities (Beck et al., 2019). In this regard, computational fluid dynamics programs are suitable programs that assist the food facilities in anticipating the movements of airflow within the facility (Oliveira et al., 2020), which also helps improve ventilation systems and enhance sanitation programs (Skåra & Rosnes, 2016).

Airborne pathogens have been found in the air of produce packing houses (Cevallos-Cevallos et al., 2012), poultry plants (Kwon et al., 2000), pork production environments (Pearce et al., 2006), and turkey production environments (Harbaugh et al., 2006). Additionally, airborne pathogens and endotoxins were found in two herb processing plants (Dutkiewicz et al., 2001). Current disinfection methods include chemical fogging, ozone, and hydrogen peroxide. Numerous oxidizing agents with solid antimicrobial activity have been evaluated to disinfect the air of food plant environments, and most of them are suitable sanitizers. The most common sanitizing agents used are chlorine dioxide (ClO2), organic acids, hydrogen peroxide (H2O2), and ethanol (Hoehn et al., 2010; Tuladhar et al., 2012). Typically, gaseous disinfectants provide advantages over liquid disinfectants because they are more easily spreadable and can reach difficult areas (Tuladhar et al., 2012; Morino et al., 2011; Yeap et al., 2016). Therefore, fogging, in this case, can enhance the application of sanitizers to reduce airborne microorganisms more efficiently than general liquid sanitizers. Hedrick (1975) tested the application of fogging and found that fogging with chlorine fog reduces airborne counts in the environment. However, the application of fogging was found to be less effective than other disinfection methods such as UV irradiation or ozone (Oliveira et al., 2020). The application of hydrogen peroxide leaves no chemical residue in treatment areas since it decomposes to water and oxygen. It can be used as a liquid or a gas, alone or in combination with heat, as high temperatures enhance its antimicrobial activity (Oliveira et al., 2020). Hydrogen peroxide fogging has been used to minimize pathogens in contaminated environments and surfaces (Oliveira et al., 2020).

Typical disinfection systems may not be sufficient to control potential airborne microorganisms in the food processing environment. Therefore, assessing emerging and innovative disinfection technologies for controlling airborne food processing facilities is necessary. Novel and new approaches for reducing and inactivating airborne microorganisms have been evaluated for food processing plants, including UV irradiation, carbon nano-tube filtration, and electrostatic field (Liang et al., 2012). UV irradiation is a potent treatment that inactivates microorganisms by damaging the molecular bonds in DNA (Brandl et al., 2014). A study found that shortwave UV radiation (254 nm) reduces microbial levels in the air and on surfaces (Bintsis et al., 2000). However, the efficacy of UV irradiation depends on different parameters, including UV intensity, exposure time, location of the lamp, and air

movement and airflow. UV irradiation disinfection has been widely used in medical and veterinary operations to decontaminate the air, surfaces, and equipment (Memarzadeh et al., 2010; Rutala & Weber, 2011). This disinfection treatment has several advantages over chemical sanitizers, including the absence of chemical residuals in the environment or surfaces, instantaneous and specific biocidal action, ease of use and installation, no maintenance required, low costs, and no chemical hazards (Brandl et al., 2014). Thus, UV technology is one of the most promising technologies as a control measure for airborne organisms.

Ozone is another emerging disinfection approach that can be used to control airborne microorganisms in food processing environments. Ozone possesses powerful antimicrobial effects and inactivates microorganisms by oxidation of nucleic acids and critical cell elements, such as glycolipids, glycoproteins, sulphhydryl groups, and enzyme amino acids (Burfoot et al., 2007). It has been widely used for water disinfection and is tested for air disinfection. The application of ozone is not new. Ozone has been used in many food processing plants, including meat, poultry, eggs, fish, fruits, produce, and dry ingredients, to inactivate different microbial contamination (Pirani, 2011). In the food industry, ozone is mainly used to disinfect environments and water. It has high penetration ability and high reactivity and leaves no toxin byproducts (Pirani, 2011). These properties make ozone effective against most microorganisms and an attractive disinfectant agent to control microorganisms in the food industry. Additionally, ozone can be used as a gas or liquid in water as it has gaseous or aqueous phases. Besides its potent antimicrobial effect, the application of ozone is not expensive compared to other treatments. Thus, ozone may become an alternative disinfection of chlorine, chlorine dioxide, hydrogen peroxide, and others (Weavers & Wickramanayake, 2001). However, ozone treatment has specific limitations, including its efficacy depending on concentration, temperature, contact time, and the targeted organisms. At high concentrations, it may cause the oxidation of food ingredients and can affect the human respiratory tract as it produces toxins (Oliveira et al., 2020). Therefore, it is suitable to use as a combined treatment with other treatments to minimize the risk of its toxicity.

Due to its unique antimicrobial properties, nanoparticle application is another promising technology for controlling airborne contamination in food processing plants. For this purpose, carbon nanotubes are coated with an antimicrobial using an electrospray system. These coated nanoparticles are used in air filters to enhance their efficiency as antimicrobials. Nanotubes and nanoparticles can be combined to form hybrid nanoparticles that settle onto the air filter medium. The hybrid nanoparticles form dendrites on the surface of the filter, making filter efficiency higher than those of original nanoparticles or nanotubes alone. The application of nanotechnology for controlling airborne contamination has been tested by Hwang et al. (2015), and the results showed a promising use for controlling airborne in indoor air environments. The above-mentioned controlling measures are typically used as a single treatment. However, hurdle approaches using a combination of two or more treatments enhance the efficacy of treatment compared to a single one.

Factors Affecting Levels of Airborne Microorganisms in Food Processing Environments

Hygiene, safety, and safe production are top priorities in food processing plants. During production, food may be subjected to bio-contamination. Among microbial vectors, air is considered an important potential source of microorganisms, including pathogens (Masotti et al., 2019a, b). Microorganisms may be responsible for the upsurge of food-related illnesses or food spoilage. In food processing plants, contamination via air, surface contact, and personnel are microorganisms' major routes of food recontamination (Fig. 19.1) (den Aantrekker et al., 2003). In food processing areas, air must be controlled and attain minimum standards. However, environmental air with specific quality factors such as temperature, humidity, dust, and microbial content are generally required to produce particular products. For example, in food industries, the zone where food products' chilling is carried out is mainly run at 10–12 °C to maintain the chilling temperature before packaging food products. Once the product is packaged, it is more difficult to chill it if it is above the required temperature (Brown & Wray, 2014).

Moreover, for high-care and high-risk areas, the main objective of an air handling system is to provide adequately filtered air at the right temperature and humidity and at a slight overpressure to prevent the ingress of air from external and uncontrolled sources. In addition, in cereal milling operations, control of airborne dust also plays an essential role in workers' health and in reducing the risk of explosions (Brown & Wray, 2014). Moreover, environmental or air quality in dairy farms also plays a vital role in the food safety of the dairy industry because it may influence the microbial communities in milk. The bacteria or microorganisms in different dairy farm areas influence this environmental quality, using air as a dissemination vehicle (Quintana et al., 2020). For example, in the cheese-making process, the hygienic quality of milk that will be used for the elaboration of unpasteurized cheese is essential because air is an important source of contamination with microorganisms being transported through it, affecting the properties of the final product (Albenzio et al., 2005). Therefore, special attention should be given to the possible contamination routes through the dairy farm environment.

Factors that affect the dairy farm environment include temperature, relative humidity, ventilation, dust, and livestock housing. Temperature plays an essential

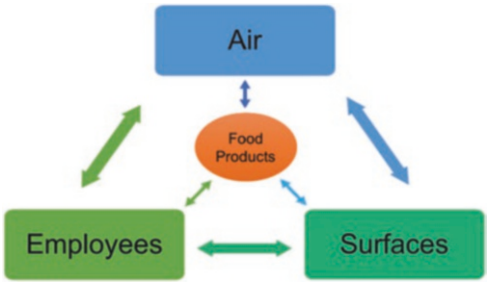

Fig. 19.1 Major routes of microbial contamination and their interactions in the food processing plant

role in a dairy farm environment, especially when temperatures are high, because a proper selection of temperature range improves the quality of the air and, thus, animal welfare (Quintana et al., 2020). Moreover, the appropriate relative humidity level in the environment should be considered to avoid the propagation of microorganisms on the farm. In addition, proper ventilation should be provided on the farm to prevent environmental pollution (Lange et al., 1997).

Effect of Temperature on Air Quality Temperature always plays a vital role in the environment of dairy farms because it is closely related to the welfare of the animals. In the last few years, the average global temperatures have risen by up to 4 °C in some parts of the world due to climate change. These temperature increases have had an impact on livestock farming systems as well as human and animal health (Marino et al., 2016). The effect of temperature on dairy farms is determined by two factors: location and season. One study observed differences in the concentration of microorganisms in the environment depending on the season (Vissers et al., 2007) or meteorological factors. Sanz et al. (2015) observed a seasonal effect on the number of isolates of bacteria (*Escherichia coli*) in the air. They observed a double concentration of bacteria in the hot season (summer) compared to the cold season (winter). They also observed the influence of the time of day on the concentration of microorganisms. Similarly, Popescu, 2011 found a positive relation between the increase in temperature and the increase in bacteria population in the environment, both in the morning and in the evening. As a result, there are seasonal and daily variations exist that cause the bacterial count to increase in the hottest seasons and during the hottest hours of the day. Furthermore, more microorganisms were detected in the air during the summer study. According to Dungan et al. (2011), environmental microorganisms are positively correlated with air temperature but negatively correlated with humidity and solar radiation.

Proper farm building design protects against climatic conditions, relieving animal stress and avoiding increases in respiration rate and exchanges between the animal's body surface and the environment, contributing to air pollution (Caroprese, 2008). The appropriate temperature is determined by the farming system. For example, in dairy cattle, the temperature should be maintained between – 5 °C to 22 °C for animals; however, this condition may vary depending on the animal's physical condition, available resources, and environmental factors. Sevi et al. (2009) suggested a range of air temperature from 5 °C to 20 °C for efficient production in small ruminants.

Effect of Humidity on Air Quality Relative humidity is a vital factor affecting respiratory damage. Because of this, it always plays an essential role in human and animal health building. Infectivity of pathogens found in the environment depends on the humidity level, due to which humidity is also critical to the welfare of animals (Xiong et al., 2017). Moreover, humidity may depend on other factors such as air distribution, ventilation, and temperature. For example, poor ventilation will occur without good air distribution, the temperature will fluctuate from its optimum range, and relative humidity will be affected, influencing the count of microorganisms

and molds. Therefore, it has been concluded that specific space in the stables is necessary for each animal to secure the correct relative humidity (Sevi et al., 1999). If living space is reduced, the concentration of pathogenic microorganisms in the environment increases. For dairy cattle, the relative humidity is between 55% and 75%; for small ruminants, around 70% relative humidity is recommended (Sevi et al., 2009). However, these values match the optimum criteria for the survival of most bacteria and fungi, 55% - 75% (Xiong et al., 2017). In addition, humidity control is necessary to minimize the risk because low humidity level negatively affects the collection of microorganisms from the environment, possibly due to their lower presence (Wilson et al., 2002). Moreover, another study found a positive correlation between the increased humidity of the air and fungi (Popescu, 2011). However, Tang (2009) observed a complicated relationship between airborne bacteria and relative humidity.

Conclusion

Air is recognized as a potential and important source of contamination in food processing establishments. Airborne microorganisms are suspended in droplets as bioaerosols. These bioaerosols may be transported from contaminated areas to clean areas in food facilities, causing food safety issues, reducing the shelf life of food products, and causing economic losses (Oliveira et al., 2020). Airborne microorganisms can originate from different sources, including ventilation systems, food production systems, raw ingredients, activities, water spraying and sanitation, and worker activity. Monitoring and timely identification of airborne microorganisms in the food processing environment are essential steps to control and prevent airborne contamination. Two methods of air sampling (passive and active air samples) are used. In active air sampling, various air samplers are available for collecting air samples from food plant environments. These air samplers have advantages and disadvantages, including impingement, impaction, cyclonic separation, filtration, and thermal or electrostatic precipitation. Air samples are analyzed to determine the concentration of bioaerosols in the air of food plants using direct culture methods or rapid methods, such as molecular and immunological approaches. To this end, the first step to controlling airborne microorganisms is to understand the level of airborne organisms in the air of food processing environments through continuous monitoring and sampling of the air. Then, maintaining good plant hygiene and sanitation is essential. Different disinfection measures are already in place for controlling airborne contamination, such as chemical fogging, hydrogen peroxide, and other chemical disinfectants. The application of many emerging and promising technologies has also been evaluated for potential use to control airborne contamination in food processing environments. However, many factors affect the level of airborne microorganisms in food processing operations and should be considered when developing new control measures.

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