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

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## 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:<br>A card containing multiple sets of tenfold serial dilutions media that mimics MPN experimental design.<br>Fluorescence reading to observe growth | APC<br>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<br>Uses Abs-labeled PdNPs rather than colloidal gold nanoparticles<br>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.<br>Label-free analyte | 2S albumin – Coa a 14 gene | Hazelnut | Moreira et al. (2023) |
| Aptamer-modified carbon dots | Fluorometric sandwich biosensor<br>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<br>Uses fluorescent dye rhodamine B loaded in nanoporous anodic alumina support<br>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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