**B. N. SEAL JOURNAL OF SCIENCE**
**ISSN:** 0975-5624
**PEER-REVIEWED JOURNAL**
https://bnsealjournal.ac.in/

**REVIEW ARTICLE**

**Received: 15-03-2026**
**Accepted: 02-04-2026**
**Published: 08-04-2026**

**Citation:** Choudhury A, Das SK, Chakraborty A, Mangar P. Biofilm and the Food Industry: Risks, Challenges, and Future Perspectives. B. N. Seal Journal of Science 2026, 14:20-28. https://doi.org/10.5281/zenodo.19472957

**DOI:** 10.5281/zenodo.19472957

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Email: *preetimangarsu@gmail.com*

**Funding:** None

**Conflict of Interests:** None

**Published by:**
Office of the Principal,
Acharya Brojendra Nath Seal College, Cooch Behar, West Bengal, India-736101

# Biofilm and the Food Industry: Risks, Challenges, and Future Perspectives

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**Abstract:** Microbial biofilm in food processing environments pose a significant threat to food safety owing to their persistence, resistance to sanitization, and role in contamination. This review concentrates on the mechanisms of biofilm formation, the impact of environmental and surface-related factors, and both existing and novel strategies for biofilm management in the food industry. Recent research indicates that microorganisms associated with biofilm display enhanced resistance to traditional disinfectants, primarily due to the protective extracellular polymeric matrix and quorum-sensing-mediated synchronization. On comparison it was observed that conventional chemical sanitizing agents are mostly insufficient; however, integrated methods that include enzymatic treatments, biosurfactants, quorum-sensing inhibitors and innovative technologies such as cold plasma, ultrasound, and pulsed electric fields demonstrate improved efficacy. Though biofilm is advantageous in industrial fermentation, their harmful impacts such as long-term equipment damage, degraded product, and public health risks are more common in food processing systems. In general, this review highlights that good biofilm management needs a technology-driven, multi-targeted approach, better hygiene practices, and a better understanding of biofilm ecology. Future research should focus on scalable, cost-effective, and sustainable strategies to reduce the risks associated with biofilm in the food industry.

**Keywords:** Biofilms, Food Safety, Quorum Sensing, Extracellular polymeric substances, Resistance, Sanitization

## Introduction

Microorganisms are universally present in the food processing environments, where they mostly exist as in structured microbial communities embedded in autogenous extracellular polymeric substance (EPS) matrix. In the food processing industries, biofilm deposition on equipment and contact surfaces has evolved as a major concern due to implications for food safety, product quality and industrial efficiency. The persistent nature of biofilm is augmented by favourable environmental conditions such as moisture, nutrient availability, and surface characteristics which allows microorganism's metamorphosis from planktonic to sessile forms. Post establishment, biofilm exhibit enhanced resistance to traditional sanitization methods. This biofilm further act as reservoirs of spoilage organisms and foodborne pathogens. This leads to reduction in shelf life, product contamination, economic losses and public health risks, mainly due to their growth in inaccessible areas in processing systems.

Apart from the negative impacts, biofilm have valuable roles in industrial fermentation and bioreactor- based production systems. Although extensive research has been carried out, current literature remains uneven, often describing individual mechanisms and isolated control strategies. Furthermore, novel recent approaches such as quorum sensing inhibition, enzymatic treatments and cutting-edge physical technologies have not been sufficiently incorporated or critically assessed in terms of real-world applicability in food processing environments.

Therefore, the objective of this review is to provide a complete and integrative summary of biofilm formation, the associated risks, and control mechanism within food industries. In comparison to earlier research, this review focuses on the amalgamation of traditional and novel biofilm management techniques while evaluating their feasibility, shortcomings and future prospects.

## Biofilm and Food Industry

Food industries generally face serious consequences due to the presence of biofilm in food processing environments, which may lead to economic losses. So, to improve and guarantee the quality and the safety of the food products, one should deeply understand the ecology of biofilm [1]. Biofilm can be defined as a microbial community that adheres to a solid surface by producing an extracellular polymeric matrix on which other associated microbes are embedded [2]. The microbes present in the biofilm matrix generally coordinate with each other with the help of a special cell to cell communication mechanism, known as “Quorum Sensing” [3]. The biofilm concept was first described by Antonie van Leeuwenhoek during observing teeth surfaces by using a primitive microscope in 1684 [4]. Matrix enclosed microbial communities which are adherent in nature and are embedded in a matrix of extracellular polymeric substances are called biofilm [5]. Biofilm can be composed of one or more than one species of microorganisms or even of mixed species of microbes [6]. Frequently in food industries, surfaces and equipments are colonised by the microorganisms that form biofilm. In most of the cases, this is a challenge and concern as biofilm formed by harmful, spoilage and pathogenic microorganisms can deteriorate the quality of food products reducing the shelf-life period of the unpacked and packaged food products. This also reduces the effectiveness of the food processing strategies. On the other hand, if the biofilm is formed by beneficial and non-pathogenic microbes, then it can be used to effectively increase the yield of the desirable product and can also be enhanced the quality and shelf life of the food product. According to some research, the fact is confirmed that warm, wet environments encourage bacterial growth. Food processing environments often contain several conditions that can favour the growth of microorganisms, potentially increasing the risk of foodborne illnesses. Sanitation and cleanliness are extremely important in such areas to minimise such risks and prevent any type of food contamination. But sometimes, even after maintaining all possible hygienic conditions, some harmful microorganisms aggregates and form biofilm in the extreme corners and gaps of the food processing equipments which may not be visible through our naked eyes or even after keen observation [7].

## Biofilm Formation and Food Processing

Microbes and their transition from planktonic stage to sessile stage usually occurs in a series of steps [8]. Stages of biofilm formation are as follows- a. Formation of surface conditioning films, b. Attachment of cells, c. Microcolony formation and development of biofilm and d. Redistribution of biofilm (dispersion and recolonization). The stages of biofilm formation have been depicted in Figure 1.

Accumulation of organic and inorganic matter from food products plays a key role in altering the physicochemical properties of the surface (surface free energy, hydrophobicity and electrostatic charge). This plays a very important role during initial attachment of single microbial cell before forming biofilm [9]. With the help of organic film, which acts as an attractant for the bacterial chemoreceptors, the bacteria forms biofilm by their chemotactic movement [10]. Cell surface appendages such as pilli, fimbriae, flagella play an important role in cell attachment process during biofilm formation [11]. Some physical forces like Vanderwaal’s forces, electrostatic interaction and some other factors are responsible for the bacterial adhesion to the surface. The attachment of bacterial cells to the surface is known as adhesion and the attachment of cell to cell is known as cohesion [12]. Increased surface hydrophobicity is responsible for the reduced repulsion between bacteria and the surface at the stage of biofilm formation [13]. Generally, biofilm is formed by irreversible attachment. After irreversible attachment, bacteria grows and multiply into microcolonies, which enlarges into a fully developed biofilm on the surface. This stage is often known as maturation. Expression of proteins like Quorum sensing (QS) protein, membrane proteins, adaptation and protection proteins and those associated with metabolic cycles, etc are also stage specific during biofilm formation [14]. Bacterial cells gradually enter the sessile stage from planktonic stage at this point. The phenomenon of dispersion of bacterial cells from the matured biofilm is known as sloughing, dispersion, redistribution, seeding, or recolonization. These released cells then further colonise to other suitable surfaces and initiate the process of biofilm formation [15]. Biofilm dispersion can be controlled by stimulating lytic enzymes that hydrolyze the exopolysaccharide matrix responsible for holding bacterial cells together in the biofilm [16].

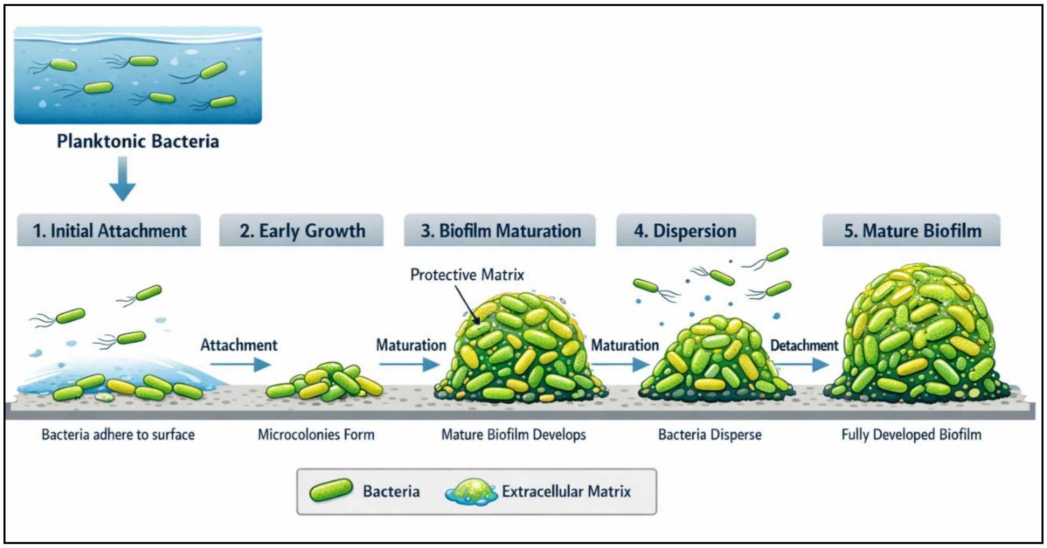


**Figure 1:** Stages of Biofilm formation

**Biofilm and Environmental Factor**

Biofilm forming bacteria, their physicochemical properties and the surface, both plays a crucial role in the initial attachment process of biofilm formation [17]. The nature of the substrate is considered to be a primary factor for biofilm formation. Water is the most important ingredient for biofilm formation. Bacteria attaches its external appendages to the surface to initiate biofilm formation. Generally, rough, corroded and nutrient rich surface is preferred by the bacteria to form biofilm. Substrate type varies from microbes to microbes [18]. The changes in pH is a very effective method to kill bacteria. Generally, bacteria generate proton motive force by extruding the protons from cytoplasm via membrane-bound proton pumps. The proton motive force will increase the proton influx passively which will make the regulation of cytoplasmic pH difficult for the cells. Some adaptations like Exopolysaccharide (EPS) excretion in bacteria may help them to tackle the fluctuating pH related problems. Exopolysaccharides (EPS) regulate pH by forming protective ion exchange barrier which creates microenvironment for the thriving bacteria against extreme environmental pH changes [19]. Mostly, optimum pH for EPS production is 7 but may vary from species to species [20]. Biofilm are viscoelastic materials, which are able to dissipate energy arising from external force and can withstand external mechanical stress [21]. Viscoelastic behaviour refers to the rheological and adhesive properties of biofilm. Biofilm's viscoelastic behaviour is directly proportional to their characteristics of recalcitrance. Environmental factors are responsible for the formation or breakage of biofilm but temperature and nutrient availability ensure the attachment/detachment of bacteria to the surface [22]. Nutrient metabolism is mainly triggered by reaction rate of enzymes. Enzyme activity is temperature dependent that can affect the growth of bacterial cells. The viscous nature of the biofilm on the surface is mainly enhanced by the temperature [23]. Through ionic interactions and ion exchange mechanisms, the Extracellular polysaccharide (EPS) present in biofilm interacts with ions in the water and boost ion adsorption reactions in biofilm. Biofilm use these metal ions as nutrient ions [24]. Signal molecules like N-acyl Homoserine lactone (AHL) molecules, oligopeptides, etc helps in increasing the total biomass of bacterial cells and induces rapid recovery of damaged parts of the biofilm [25]. Surfactants or biosurfactants are considered to be secondary metabolites which helps in various biological functions like nutrient uptake, cell-surface modification, cell-motility and development of biofilm [26]. Secondary metabolites (rhamnolipids) help in managing the growth of stable biofilm. Impact of environmental stimuli on biofilm formation or shear stress is an important environmental factor that is associated with the compactness and porosity of the biofilm. Higher the shear stress, denser the compactness and lesser the size of pore of the biofilm [27]. High nutrient concentration favours planktonic bacterial growth. Sometimes high nutrient concentration also increases the adhesiveness of the biofilm forming bacterial cells [28].

**Biofilm and its Effect in Food Industry**

Biofilm can be formed by one or more species or even with mixed species. Some microbes are beneficial and some are harmful for us as well as for the environment. So, it can be said that biofilm have some beneficial aspects too in the industry. It generally shows positive effect in the environment when it is composed of some beneficial microbes and shows minimum adverse effects. Maintenance of water quality is generally done with the help of biofilm not only in industries but also in natural environment [29]. It has many benefits in biotechnological industrial applications as well. Due to their capability of self-immobilization with high concentration of mass within EPS, they show high resistance to toxic compounds and show long term activity which leads to continuous processing with high stability. Biofilm when used in reactors, are highly cost effective and works efficiently in product recovery. Biofilm reactors due to its several advantages, is considered to be economically beneficial for the industrial world [30]. Biofilm is used in food industries for production of various value-added products like organic acids (acetic acid, lactic acid, succinic acid, fumaric acid), polysaccharide, ethanol, butanol, vinegar etc. It can also be used in the process of fermentation where the cells can be recycled for the food processing without the need for re-inoculation of the culture. Mostly, biofilm which are composed of single species are widely used in food industry. *Zymomonas mobilis* biofilm are generally used for ethanol production. Acetic acid bacterial biofilm is grown on beechwood shaving to obtain vinegar from ethanol, which is further used to produce acetic acid finally. *Lactococcus lactis* biofilm is grown on cotton fabric to produce nisin [31].

Besides having many benefits of using biofilm reactors in industry, it has some limitations too. Excessive EPS sloughing leads to the difficulty in downstream processing and purification of product. Excessive cell growth will form thick layer of biofilm which will cause the blockage of the process where there will be no optimum mixing. There will be several more limitations which will reduce the efficiency of biofilm reactors in the industry. The thick layer of biofilm will also be responsible for the limitation of substrate and oxygen for the inner most of the microbes present in the biofilm which will affect the aerobic wastewater system. Additionally, the product will not efficiently diffuse through the thick layer of biofilm, and cannot be recovered properly, which may affect the cells present in the biofilm due to the presence of toxic compounds trapped in the biofilm products. [32]. The property of bacterial adhesion of biofilm to the surface has become a very significant problem in industries such as dairy, oil, paper, food, hospitals, etc. The formation of biofilm makes the cleaning process extremely difficult. Biofilm is responsible for many detrimental effects in the industry like unpleasant odour, pipe blockage, corrosion, spoilage of product, reduction of efficiency of production, infection, equipment failure etc. It can cause biocorrosion of water pipes due to its presence in the water flowing through the pipes, resulting in the change of quality of water [33].

Biofouling is another negative effect of biofilm which generally blocks the membrane filter, mostly observed in wastewater treatment carrying contamination [34]. Biofilm formed by sulphate reducing bacteria plays an active role in showing its detrimental effects in oil industry resulting in oil spoilage, pipe corrosion, filtration equipment blockage [35]. Many microbes can easily form biofilm on food products or raw food materials due to the presence of EPS layer which helps them in food metabolization, cell protection, signalling and also protects against desiccation, disinfectants, toxic chemicals resulting in causing foodborne diseases [36].

**Biofilm Problem Area and Food Processing Facility**

Biofilm is an emerging concern in food industry due to its presence in food processing environments. Mostly mixed species biofilm community are observed in such environments. Generally, biofilm is most often observed in a thick layer in areas which are hard to reach during the cleaning process like wall corners, drains, storage tanks, dead ends of crevices, pipes, gaskets etc. Pathogens like *Staphylococccus putrefaciens*, *Pseudomonas* sp. generally colonises due to cross contamination and successfully contaminates the food product. It is very difficult to completely eliminate and clean this contaminated microbial biofilm in such poor accessible areas. Due to the presence of contaminated pathogens in food processing environments, many issues like mechanical blockages, deterioration of product quality, damage of polymeric and metallic components in the areas of processing, food spoilage is observed. This is a potential source of public health risks and also leads to reduction in the efficiency of the technologies used in the processing areas. These issues eventually become responsible for the deteriorating economy of the food industries [37]. Dairy industries also face similar problems due to presence of biofilm in the food processing areas like feeding units, packaging machines, door mats, floor drains, etc. Other researchers have also reported about the role of biofilm in the spoilage of meat products. However, only using detergent or disinfectant cannot completely eliminate these attached microbes from the food products. Disinfectants along with use of peracetic acid or hypochlorous acid while scrubbing in the cleaning process may reduce the possibility of formation of biofilm in such areas of food processing environments. Additional factors such as temperature control, nutrient availability, water availability, maintenance of proper hygiene and cleanliness, design of equipments and equipment cleanability are some of the most important factors in controlling biofilm. More effective ways by using antimicrobial agents against bacteria are appreciated but it is generally used for planktonic cells in batch culture in which the cells are free and distinct. Biofilm consist of multiple cells and forms a thick layer. The ecology and characteristics of the biofilm should be more carefully understand to develop a better way to control biofilm.

**Biofilm Control and Food Industry**

There are many control methods which are used in food industries to reduce the microbial biofilm. Under chemical treatments to control biofilm, mostly sanitizers are used which may be time or concentration dependent. In food processing industries, sanitization of equipments becomes extremely important to prevent cross contamination. Most commonly used sanitizers are chlorine-based sanitizers. Nowadays, due to extreme use of such sanitizers, microbes like *Salmonella enterica* have acquired resistance against these kinds of sanitizers. Gaseous $ClO_2$ are more effective against *Bacillus cereus* endospores which resides as a biofilm on the steel surfaces in food processing industries. The most commonly used sanitizer is aqueous $ClO_2$ in food industry [38]. In spite of ozone being a toxic gas, it possesses oxidizing ability which destroys the cellular envelopes of the microbes and also prevents moulds from over growth ion dairy industries. NaOCl can effectively eradicate biofilm formed by pathogens like *Staphylococcus aureus* and *Salmonella enterica* which resides on the stainless steel and polypropylene surface in dairy industries [36]. $H_2O_2$, with the oxidising ability of its free radicals, can destroy the microbes in food industry. It does not show any toxic side effects. Peracetic acid is formed by the combination of acetic acid and $H_2O_2$. Peracetic acid is a strong oxidant which is used effectively in water pipe treatments against *Listeria monocytogenes* and *Staphylococcus aureus* [39].

Enzymes are often said to be ecofriendly counter measures which prevents biofilm formation as they have low toxicity and are biodegradable in nature. In case of applications in food industry, they are used in industrial detergents. Cellulase is a type of enzyme which decomposes cellulose and some related polysaccharides. It is the main constituents of plant cell wall and vegetable fibres. These are generally used in industrial detergents in food industry. Treatment of cellulose and cetyltrimethylammonium bromide can successfully prevent biofilm formation in seven strains of *Salmonella enterica* from meat processing surfaces [40]. Protease, which can also be called peptidase or proteinase, is a type of enzyme which catalyses proteolysis and breakdown the proteins into single amino acids or smaller polypeptides. Because of their lower substrate specificities, they are considered to be highly efficient to control organic based biofilm. After the partial degradation of biofilm by proteases, it can be completely eliminated by further mechanical treatments or sanitizers, to which these are more sensitive. On polystyrene surfaces, it can efficiently remove biofilm formed by *Staphylococcus aureus* but mixture of protease, amylase and cellulose is required to eliminate the biofilm formed by *Pseudomonas aeruginosa* [41]. Glycosidases are a type of enzymes that breakdown complex carbohydrates into simpler ones. Glycosidases like amylase, dextranase, pectinase etc are the first option to control biofilm formation. Pectin methyl esterase is an enzyme that can successfully prevent biofilm formation in bioreactors [42]. Microbes like *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes* secrete some exogenous DNA during the formation of biofilm. In such cases, bovine DNases can be a good option to destroy the multicellular structures. Combination of DNases with some physical, chemical treatments and different types of enzymes can enhance the process of elimination of biofilm [43].

Biosurfactants are surface active natural biomolecules which are generally produced by microorganisms. It has a wide range of application in the modern world. It has unique properties like low toxicity and specificity. They are generally used to modify the hydrophobic characteristics of bacterial surface which prevents bacterial adhesion and surface binding.

Lichenysin is a non-ribosomal lipopeptide that is cyclic in shape and is produced by *Bacillus licheniformis*. This is used in food industry to eliminate the microbial surface binding of microbes like *Camplyobacter jejuni, Candida albicans* [44]. Generally, surfactants are produced by *Bacillus amyloliquefaciens* and *Bacillus subtilis*. These molecules decrease the surface tension and prevent surface binding molecules. It also inserts itself into the cellular membrane and changes the membrane permeability eventually resulting in cellular lysis [45]. There are many signalling pathways which are used by microbes forming a biofilm for communication. Most common pathway is known as quorum sensing. Inhibiting this pathway will also help in preventing the biofilm formation at a very early stage. Quorum sensing helps in regulation of gene expression in microbes and also uses many signalling molecules like peptides, acyl homoserine lactones, etc. Quorum sensing inhibitors (QS inhibitors) quenches the mediators of QS system (QQ etc). These compounds create less selection pressure, hence the chance of development of resistance against these microbes is also very less. There were many strategies which solely focused in inhibiting the QS systems like inhibitors binding to QS receptors, degradation of enzymes related to QS signals, small RNA (sRNA) post transcriptional control, QS signal biosynthesis inhibition [46]. Paraoxons enzymes hydrolyses lactone ring of AHL eventually blocking the QS and killing the microbes. These are isolated from mammalian sera.

**Biofilm Formation Control and Food Technology**

In today's busy world, much is dependent upon technology for reduction of time consumed, efficient work and better economic gain. Many industries have switched to advanced food technologies to control biofilm formation [47]. The plasma treatment has the ability of low penetration, so it is preferred for biofilm control. Some factors are needed to be controlled during this treatment like the measurement of the distance between surface and plasma, microbial load's thickness, which depends upon the type of surface. Biofilm's inactivation depends upon different characteristics of plasma like setup, frequency, exposure mode, operating gas, exposure time, plasma intensity in voltage etc. This technique is effective against many microbes like *Escherichia coli*, *Salmonella enterica*, *Salmonella typhimurium*, *Pseudomonas fluorescens* [48]. Di-electric barrier charges (DBD) is a plasma source which is efficient in ACP (atmospheric cold plasma) [49]. After many researches, it has been concluded that ACP in combination with other surface treatments (hydrogen peroxide, chlorhexidine, polyhexanite, sodium hypochlorite) is highly efficient in controlling biofilm. Ultrasound assisted technologies has two known frequencies- low (16 kHz−1 MHz) and high (>1 Mhz) [50]. This technology is used in combination with other inactivation methods to disrupt biofilm. When it is combined with mild heat and electrolysed water (slightly acidic), it could successfully inactivate *Bacillus cereus* on the surface of green leaves. Moreover, this treatment along with peroxyacetic acid showed effective reduction of biofilm produced by *Cronobacter sakazakii* [47]. Pulsed electric field is a technology used in food processing industries applies high voltage for a short period of time. It increases the cell permeability resulting in the damage of cell membrane [51]. If the potential is high enough, then electroporation also gets produced. In case the pores are not repaired within a stipulated time, then it may result into cell death. In combination with additives like EDTA, pulsed electric field effectively inhibits the biofilm formation by *Salmonella* in whole liquid eggs [52]. Due to its high efficacy against biofilm forming microbes, it is widely used in food processing industries. High pressure processing, is an another commonly used alternative to conventional methods used in early times for food processing. The advantage of this method is its high effectivity against biofilm forming microbes and its toxic enzymes and its non-interference in the nutritional properties of the food. In combination with other pathogen inactivation methods, it can more effectively work and also can also contribute in targeting specific inactivation of pathogen, eventually resulting in prevention of biofilm formation [47].

## Conclusion

Biofilm in food processing industries have a dual role, both as a contaminating source leading to economic loss as well as valuable agents of fermentation and bioprocessing. This review accentuates that formation of biofilm is universal and highly resistant to conventional sanitization methods, rendering single chemical control methods insufficient and unsustainable. Simultaneously, the valuable role of biofilm requires further research with localized precise management rather than extensive elimination.

Therefore, effective management necessities integrated and multi targeted strategies that include chemical, physical and biological interventions. Novel and recent tools like enzymes, biosurfactants, cold plasma, quorum sensing inhibitors, ultrasound, pulsed electric fields and high-pressure processing not singly but in combination might prove to have a great potential in managing the biofilm. These processes not only work in synergy but prove to overcome limitations such as poor penetration, resistance development and operational constraints. The other factors like equipment design, ease of cleaning and biofilm resistant materials while designing food processing units should be considered.

Despite extensive research, significant gaps do remain in transitioning novel strategies into scalable real-world applications. Furthermore, the lack of integrative approaches in current biofilm management practices is an impediment. In the near future, development of multi targeted integrated management system with early-stage detection and monitoring of biofilm could be used. A deeper level of research and understanding of microbial ecology and communication could help to disrupts biofilm at molecular level. Additionally, further research on the exploration to optimize beneficial biofilm while minimizing contamination risks and evaluation of long-term efficacy and safety of biofilm management techniques should be incorporated.

Therefore, proper coordination of technical, economic and regulatory gaps is essential for next generation biofilm management. A major shift from reactive cleaning to proactive and system level control could prove to better to safeguard product quality and protect public health.

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