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Biofilm: A hidden menace in the dairy industry

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Abstract
Biofilms are structured microbial communities that adhere to surfaces and pose significant challenges in the dairy industry by contaminating milk and milk products, reducing equipment efficiency, and compromising food safety. Their formation involves microbial adhesion, extracellular polymeric substance production, and the establishment of resistant colonies. Biofilms harbor pathogenic bacteria like *Listeria monocytogenes*, impacting product quality and safety. Control strategies include effective sanitation, material selection, and quorum-sensing inhibition. While biofilms are a concern in dairy processing, beneficial biofilms in the gastrointestinal tract contribute to gut health. Innovative solutions, such as antimicrobial coatings and enzymatic cleaners, are essential for biofilm management in dairy industries.

Keywords: Biofilm, milk, microbial, dairy industry, enzyme

Introduction
The dairy industry is a significant segment of the global food sector, producing a diverse range of perishable (e.g., butter, yogurt, and cheese) and semi-perishable (e.g., milk powder, casein) dairy products. Ensuring the quality and safety of these products necessitates stringent microbiological guidelines. Although milk obtained directly from a healthy lactating animal is sterile, it becomes susceptible to contamination at various stages, including milking, transportation, storage, and processing. Multiple sources contribute to microbial entry, and inadequate sanitization can lead to the accumulation of contaminants in processing equipment. These contaminants often develop into biofilms, which serve as persistent sources of microbial contamination in dairy products.

Biofilms are structured communities of microorganisms embedded in a self-produced matrix of extracellular polymeric substances (EPS). They facilitate microbial growth on diverse surfaces, posing a serious challenge to the dairy industry (Costerton *et al*., 2004) [29]. Unlike free-floating (planktonic) microbes, biofilm-forming microorganism's exhibit altered phenotypic characteristics that enhance their resistance to environmental stressors, including sanitizers and antibiotics (Donlan & Costerton, 2002) [5]. Their presence in dairy processing environments compromises the quality and safety of raw milk and its derived products.

With the increasing consumer demand for high-quality dairy products with extended shelf life, biofilm research has gained attention in the food industry. Modern dairy operations, characterized by automation, complex equipment, and shorter production cycles, further underscore the importance of addressing biofilm-related contamination. Pathogens such as *Listeria monocytogenes* thrive in biofilms, making their control a priority for ensuring food safety (Finn *et al*., 2023) [7].

Although biofilm formation has been recognized for centuries, its implications in food industries remain a pressing concern. The concept dates back to 1684 when Antony van Leeuwenhoek first observed microbial accumulations on dental plaque, describing them as "animicules" in a report to the Royal Society of London. In the 1940s, scientists studying marine organisms documented their ability to adhere to surfaces, further advancing the understanding of biofilms (Flemming *et al*., 2016) [8]. Recognizing the economic impact of biofilms, the U.S. National Science Foundation established the Center for Biofilm Engineering at Montana State University in 1990. Today, research on biofilms is conducted globally, with significant contributions from institutions in Denmark, England, Germany, Australia, and Singapore.

To mitigate the risks associated with biofilm formation in dairy processing, various strategies have been proposed. Meyer (2003) [15] suggested three primary approaches: (i) timely disinfection before biofilm formation, (ii) removal of established biofilms using strong disinfectants, and (iii) prevention of microbial adhesion by modifying surface materials or supplementing with inhibitors. Additionally, an understanding of microbial adhesion mechanisms and colony proliferation is crucial for developing effective interventions. The adoption of eco-friendly or "green" control strategies, such as enzymatic treatments and bacteriophage-based approaches, holds promise for sustainable biofilm management (Simoes *et al*., 2010) [28].

Given the persistent nature of biofilms and their impact on dairy product safety, ongoing research and innovative sanitation strategies are essential. Implementing targeted control measures can significantly reduce contamination risks, ensuring the production of high-quality, safe dairy products.

What is a biofilm?

A biofilm is a structured community of microorganisms in which cells adhere to a surface and to each other, often encased within a self-produced matrix of extracellular polymeric substances (EPS). This matrix consists of polysaccharides, proteins, lipids, and DNA, providing structural integrity and protection to the microbial community (Stoodley *et al*., 2004) [29]. Biofilms are sometimes referred to as biofouling or microbial fouling, indicating the unwanted accumulation of microorganisms and their metabolic byproducts on surfaces in contact with liquid media.

Biofilms are ubiquitous in nature and can form on various surfaces, including rocks and pebbles in streams and rivers, stagnant pools, water pipelines, marine engineering systems, sewage pipes, and industrial equipment. In biological systems, they are present as dental plaque on animal teeth and on plant surfaces, both internally and externally (Schwermer *et al*., 2008) [25].

Sauer *et al*., (2022) [23] define biofilms as sessile microbial communities in which microorganisms exist in close association with each other, adhering to biotic or abiotic surfaces while being encased in an EPS matrix. In simpler terms, biofilms represent a dominant mode of bacterial life, colonizing almost all surfaces in natural and industrial environments.

Biofilms and infectious diseases

Biofilms have been found to be involved in a wide variety of microbial infections occurs in the human and animal body. Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle-ear infections, formation of dental plaque, gingivitis, coating contact lenses, and less common but more lethal processes such as endocarditis, infections in cystic fibrosis, and infections of permanent indwelling devices such as joint prostheses and heart valves (Rogers 2008) [19]. More recently it has been noted that bacterial biofilms may impair cutaneous wound healing and reduce topical antibacterial efficiency in healing or treating infected skin wounds.

Mechanism of biofilm formation

In nature microorganism get attracted to solid surface conditioned with nutrients that are sufficient for their viability and growth. Initially these microorganisms get deposited on the surface, and later get attached, grow and actively get multiplied to form colony of cells. Biofilm formation is a dynamic process and different steps are involved in their attachment and growth.

Biofilm formation

The continuous attachment of the bacterial cells to the surface and subsequent growth along with associated EPS production forms a biofilm. Multilayers of bacterial cells entrapped within the EPS containing matrices develop within the biofilm. Composition of the biofilm is heterogeneous due to the colonization of different microorganisms possessing different nutritional requirements. It does not exist as a uniform layer throughout the surface. Further increase in size of biofilms take place by the deposition or attachment of other organic and inorganic solutes and particulate matter to the biofilm from the surrounding liquid phase. The interaction of various microbial populations during the initial stages of biofilm formation has a significant effect on the structure and physiology of the microbial biofilm (Kumar and Anand 1998) [13]. Mixed species biofilms are often thicker and more stable than monospecies biofilms. It is large, complex, and organized bacterial ecosystem in which water channels are dispersed providing passages for nutrient, metabolite, and waste product exchange (Sauer *et al*., 2007) [22]. Buregess *et al*., 2014 [2] determined the ability of different strains to form biofilm and produce spores and concluded that *G. stearothermophilus* strains isolated from milk powder manufacturing plant had differences in their ability to form biofilm and produce spores which also influenced the cleaning method used to control growth of *thermophilic bacilli.*

Biofilms in dairy industry

Milk is the best medium for the growth of microorganisms because of its almost neutral pH and wide variety of available nutrients. Even though milk is rich in minerals many of them may not be present in a form that can be utilized directly by bacteria. Milk contains growth stimulants such as orotic acid, which is a metabolic precursor of pyrimidine’s and which fosters microbial growth. Bacterial contamination can adversely affect the quality, functionality and safety of products produced by the dairy industry. When contamination of dairy products occurs evidence suggests that biofilms on the surfaces of milk processing equipment are a major source (Flint *et al*., 1997) [9]. Biofilms are not only a potential source of contamination, but can also increase corrosion rate, reduce heat transfer and increase fluid frictional resistance (Kumar and Anand 1998) [13]. With current trends towards longer production runs, the use of complex equipment, the automation of plants, and increasingly stringent microbiological requirements, bacterial biofilms has become a major concern to dairy manufacturers. Biofilms developed on interiors of pipes leads to clogging and corrosion especially in engineered systems. Biofilm on floors can make sanitation difficult in milk production area as they remain protected within the biofilm from the sanitizer due to EPS (Geetha and Prasad, 2011) [10] and shows inhabitant resistance to antimicrobial agents. In dairy manufacturing plants biofilms can be divided into two categories, vizbiofilms that are unique to dairy manufacturing plants which have been termed "process" biofilms e.g. those which form on surfaces (e.g. heat exchanger) in direct contact with flowing product, and biofilms which form in the general milk processing environment. The *et al*., 2014 observed that bacteria from milk tankers forms multispecies biofilms and produce heat-stable enzymes, the free peptide concentration, which indicated proteolysis, was higher in UHT milk when exposed to multispecies biofilms than in untreated UHT milk. Rysstad and Kolstad (2006) [20] showed pasteurized and extended shelf life (ESL) milk filling machine as the main source of recontamination, with the filler nozzles, aerosols, and the water at the bottom of the filling machine being of particular concern.

In dairy processing environments, the most commonly encountered bacteria belong to the genera Enterobacter, Listeria, Micrococcus, Streptococcus, Bacillus, and Pseudomonas (Wiedmann *et al*., 2000; Waak *et al*., 2002; Salo *et al*., 2006) [32, 31, 21]. These microorganisms pose significant risks due to their ability to form biofilms, which can lead to persistent contamination. Biofilm accumulation and microbial colonization frequently occur in milk pipelines, storage tanks, and milk silos (Shaheen *et al*., 2010) [26]. Additionally, fouling of heat exchangers is a major concern, as it can reduce processing efficiency and compromise product safety (Flint *et al*., 1997) [9]. Research suggests that biofilm formation on milk evaporators, followed by sloughing off into the product line, is a key factor contributing to high contamination levels in the final dairy products (Hinton *et al*., 2002; Palmer *et al*., 2010) [12, 17].

Novel methods for biofilm prevention and removal

Cleaning procedures in dairy manufacturing plants have traditionally relied on cost-effective chemicals such as caustic soda, acids, and chlorine. Most sanitization protocols have remained largely unchanged since the early 1900s. A significant advancement in cleaning technology was the introduction of clean-in-place (CIP) systems in the 1950s, a period when manufacturing plants were smaller, processes were simpler, and regulatory requirements were less stringent. Over time, modifications to these systems have mainly focused on reducing cleaning costs and protecting sensitive equipment, such as ultrafiltration membranes. However, current cleaning methods require reassessment to effectively control biofilms in dairy processing environments. While preventing biofilm formation is ideal, there is currently no universally effective method that can completely prevent or eliminate biofilms without causing undesirable side effects (Simoes *et al*., 2010) [28].

The primary strategy for biofilm prevention is regular cleaning and disinfection before bacteria can firmly adhere to surfaces (Midelet & Carpentier, 2004; Simoes *et al*., 2006) [16, 27]. Common disinfectants used in dairy processing include acidic compounds, aldehyde-based biocides, caustic solutions, chlorine, hydrogen peroxide, iodine, isothiazolinones, ozone, peracetic acid, phenolics, biguanidines, and surfactants (Bremer *et al*., 2006; Dosti *et al*., 2005) [1, 6].

Several studies have explored optimized cleaning techniques. Kumari and Sarkar (2014) [14] conducted an in-vitro model study and proposed an optimized cleaning procedure using 1.5% NaOH at 65 °C for 30 minutes, followed by a water rinse, then 1% HNO_3 at 65 °C for 10 minutes, and another water rinse for effective biofilm removal. Schlisselberg and Yaron (2013) [24] demonstrated that electropolishing the surface of AISI 316 stainless steel significantly reduced biofilm thickness, covering only 23% of the surface compared to untreated stainless steel. Additionally, Cloete and Jacobs (2001) [4] reported that pre-conditioning surfaces with surfactants can help prevent bacterial adhesion.

Given the persistent challenges posed by biofilms in dairy manufacturing, continued research into advanced cleaning techniques and surface modifications is essential to improve hygiene standards and ensure product safety.

Biofilm signaling and future perspective

Extensive research and ongoing advancements in biofilm disinfection continue to explore innovative strategies, including the development of molecules that interfere with quorum sensing (Girennavar *et al*., 2008; Pan and Ren, 2009) [11, 18]. In bacteria, cell-to-cell communication is a widespread phenomenon that regulates various physiological activities through the production, secretion, and response to small diffusible signal molecules known as auto inducers. These signaling molecules are produced at a basal level during bacterial growth and play a crucial role in both intercellular and intracellular communication. This process, known as quorum sensing, is directly linked to bacterial pathogenicity and food spoilage. Therefore, disrupting quorum-sensing signaling in food-related bacteria presents a promising approach to preventing spoilage and enhancing food safety. Quorum-sensing inhibitors or antagonists could be incorporated into food preservation systems to extend shelf life and improve overall food quality.

Biofilms pose a significant threat to the dairy and food processing industries, leading to safety concerns, economic losses, and damage to brand reputation. Despite various approaches being explored for biofilm prevention and control, there is a continuous need for innovative solutions. One potential strategy involves coating equipment surfaces with materials that reduce microbial attachment. Additionally, the design of advanced cleaners and sanitizers with enhanced biofilm removal properties could be a viable solution. For instance, fortifying cleaning agents with extracellular polymeric substance (EPS)- or protein-degrading enzymes, along with quorum-sensing inhibitors, may help disrupt biofilm formation at different stages, thereby minimizing contamination risks.

While biofilms are often considered detrimental, they can also have beneficial applications. The human gastrointestinal tract is naturally colonized by lactic acid bacteria and *Bifidobacterium* species, which form a protective biofilm-like layer that prevents the colonization of pathogenic bacteria. These beneficial microorganisms help maintain microbial balance in the gut and exert probiotic effects when consumed through fermented foods, contributing to overall health and well-being.

Conclusions

Bacterial biofilms are omnipresent in nature, and the dairy industry does not escape from the problems they can cause. In particular, biofilms formed on milk-processing equipment and other food contact surfaces act as a persistent source of contamination threatening the microbiological quality and safety of milk products, and may result in food-borne disease and economic losses. Some of the approaches which can be used for prevention and control of *biolfilm* are modification in existing CIP programmes, Use modified detergents and sanitizer, give special attention to biofilm prone areas in processing line and probably best option is to use green chemicals to reduce the environmental problems.

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