

Review Article

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Biofilm: An Overview with Respect to Dairy Industry

Suchita Bhosale*, R. J. Desale and Y. G. Fulpagare

Department of Animal Husbandry and Dairy Science, Post Graduate Institute,
M.P.K.V. Rahuri, India

*Corresponding author

ABSTRACT

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etc)

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Milk obtained from the udder of a healthy milch animal is almost in sterile condition but gets contaminated during milking, transportation, storage and processing and also due to the entry of microbes through many other sources. Insufficient sanitization and cleaning causes contaminants to accumulate in milk processing equipment which subsequently form biofilm that further become significant source of contamination of dairy products. Biofilm is aggregations of microbial cells interconnected by extracellular polymeric substances which accelerate growth on different material surfaces adversely affect the dairy industry. Biofilm formation possesses profound implications and throws a major challenge to the dairy sector where they act as the principal reservoir of microbial contamination. These lead to financial crisis by impairment of raw material and its products. It is emphasized that good manufacturing practice, good hygienic practice and hazard analysis and critical control point should be implement in dairy industry to prevent the contamination of dairy products.

Introduction

According to the changing scenario of global market, the dairy industry is considered to be one of the major food industry in world which manufactures a wide range of perishable (e.g. butter, yoghurt and cheese) and semi perishable (milk powder, casein) milk products. To maintain the quality and safety of these products, microbiological guidelines are an essential requirement. Milk obtained from the udder of a healthy milch animal is almost in sterile condition but gets contaminated during milking, transportation,

storage and processing and also due to the entry of microbes through many other sources. Insufficient sanitization and cleaning causes contaminants to accumulate in milk processing equipment which subsequently form biofilm that further become significant source of contamination of dairy products. Biofilm is aggregations of microbial cells interconnected by extracellular polymeric substances which accelerate growth on different material surfaces adversely affect the dairy industry. This polymicrobial community contains altered phenotype which differ them from planktonic microbes physiologically. It

affects the quality and safety of raw materials and their products.

What is a biofilm?

The existence of biofilm has been explored for several years in the food industry. The first documentation was done roughly 75 years back in 1943.

Biofilms are three dimensional aggregations of microorganisms attached to surfaces. Bacteria in the biofilm join together and form a protective matrix around each other. It is

estimated that up to 90% of microbial populations exist as biofilms, rather than as discrete organisms (planktonic cells) floating around in the environment.

“Biofilms are sessile microbial communities where microbes live together in association with each other on biotic or abiotic substrates which are bounded by extracellular polysaccharides, proteins, lipids and DNA.” In other words, simply, biofilms represent an important mode of bacterial life colonizing most of the surfaces in nature (Singh *et.al.* 2019) (Table 1–9).

History of biofilm

Year	Investigator	Contribution
17 th century	Antony van leeuwenhook	First examined microorganisms from his own teeth surfaces
1930	Claude ZoBell	Research on bacterial adhesion to surfaces
1978	Bill costeron	Biofilm coined
	Heukeleian and Heller	Bottle effect for marine microorganisms
	Jones et al	Used scanning and transmission electron microscopy to examine biofilms
2002	Donlan and Costerton	Given silent description of biofilm

(Patel, 2014)

Why microorganisms prefer to exist in biofilm?

Microorganisms residing in biofilms get many advantages as compared to freely swimming planktonic stage, and that’s the reason for them to prefer biofilm mode of living. Some of these potential advantages are:

Microorganisms in biofilms exhibit elevated antimicrobial tolerance and also get protected from environmental stresses such as extreme pH, oxygen, osmotic shock, heat, freezing, UV radiation, predators, and so on.

Extracellular polymeric matrix formed from the secreted exopolysaccharides (EPS)

increases the binding of water resulting in decreased chance of dehydration (desiccation) of the bacterial cells, which is a common stress condition experienced by planktonic cells.

The adherent nature of microbial cells in biofilms allows rapid exchange of nutrients, metabolites, and genetic material.

Extracellular polymeric substances (EPSs)

Extracellular polymeric substances consist primarily of polysaccharides. EPSs provide the matrix of structure for the biofilm. They are highly hydrated (98% water) and tenaciously bound to the underlying surface.

Has “water channels” that allow transport of essential nutrients and oxygen to the cells growing within the biofilm. Biofilm acts as filters to entrap particles of various kinds including minerals and host components such as fibrin, RBCs and platelets. EPSs may associate with metal ions, divalent cations other macromolecules (such as proteins, DNA, lipids and even humic substances)

Biofilm locations

Dairy processing plants
Food and beverage plant products line
Water system
Pharmaceuticals manufacturing processes
Raw materials suppliers processes
Cleaning chemicals
Steam lines
Cosmetics and nutraceuticals plants
Heat exchangers

Why study of biofilm in dairy industry is important??(Genesis)

Case Study: Several food borne illnesses occur due to consumption of milk and milk products from dairy industry contaminated with pathogens. It is clear that contamination of dairy product can occur post pasteurization. This may be as a result of cross contamination of finished product with raw product, inadequate sanitation procedures in the plant environment or inadequately sanitized equipment.

Biofilm formation in dairy industry is always noted as threat which affects the product safety and thereby resulting in food borne illness. So, it is considered as an emergent public health concern throughout the world.

Consumer demand for higher quality products with respect to their shelf life and safety.

Current trends towards lower processing runs, automation, complexity of equipment and

increased awareness of the problems caused by pathogens such as *Listeria monocytogenes* makes biofilm a concern.

Bacillus subtilis, and *Bacillus cereus* will often cause sweet coagulation and bitter taste in milk and cream and the gas producing *Clostridium tyrobutyricum* may cause spoiled texture and late-blowing in semi-hard cheeses.

Spore forming thermophilic and thermophilic bacteria are commonly found in high numbers in milk powder after 16-20 hours production time due to biofilms formed on the large internal surface in evaporators and spray dryers.

To determine density of the biofilms formed by bacteria isolated from dairy equipment.

Biofilm formation process

A biofilm is composed of complex communities of bacteria mostly with mixed species that is irreversibly attached to different surface materials. This structure is enclosed in a matrix of primarily polysaccharide material and a mix of proteins, lipids, and nucleic acids forming a single layer or three-dimensional structures (Fig. 1 and 2).

Characteristics of biofilm

Biofilms are complex, dynamic and remarkably heterogeneous structures. Different biofilms exhibit different chemical and electrical properties. Moreover the genetic expression is also different in biofilm bacteria as compared to the planktonic bacteria.

The cells are able to coordinate among each other via intercellular communication using biochemical signalling molecules.

Stainless steel, polyvinyl chloride, polyurethane are the prolific surfaces for biofilm attachment when they come in contact with food materials.

much less susceptible to antimicrobial agents than those present in planktonic state. As a result, it becomes difficult to get rid of biofilms from the contact surfaces of food. (Singh *et al.*, 2019)

The biofilm associated microbes are also

Table.1 Recent Outbreaks due to Milk and Milk Products Consumption

Month/ Year	Location	Details	Reference
April 2012	Missouri	14 sick from raw milk (<i>E. coli</i>)	(Mogha <i>et al.</i> , 2016)
April 2012	Oregon	21 sick from <i>E. coli</i> associated with raw milk consumption	
October 2011	Indiana	0 illnesses. Recall of pasteurized cheeses because of potential <i>Listeria</i> contamination	(Mogha <i>et al.</i> , 2016)
September 2011	Georgia	0 illnesses. Improper pasteurization of milk	
August 2011	Virginia	0 illnesses. recall of pasteurized cheddar cheese spread with possible <i>Salmonella</i> contamination	
March-August 2011	Pennsylvania	16 illness from <i>Yersinia enterocolitica</i> from glass-bottled pasteurized milk and ice cream	
June 2011	New York	No illnesses from pasteurized queso fresco cheese contaminated with <i>Staphylococcus aureus</i>	

Table.2

Biofilm formation Stages	Descriptions
Stage I: Initial reversible attachment	Biofilm formation -Starts attachment of plank tonic bacteria to solid surfaces. The reversible attachment is attained- between bacteria and surface through van der Waals and electrostatic forces. The direct contact with the surface material occurs via the surface appendages of bacteria such as flagella, fimbriae and extracellular polymers. During this stage cell exhibit logarithmic growth rate.
Stage II: Irreversible attachment	The biofilm grows through a process of cell division and reversible attachment. This attachment, then, differentiates to irreversible attachment with the production of extracellular polymers (EPS) by the bacteria. Motility is decreased and cell aggregates are formed then it becomes layered.
Stage III: Early development of biofilm structure	The EPS layer strengthens the structure between bacterial cells and the substratum. Over a period of time, the interactions and bonds are strengthened, making the attachment irreversible.
Stage IV: Maturation	During the maturation, the biofilm develops into an organized resistant structure to toxic chemicals and disinfectants. The irreversibly attached cells grow more by using available nutrients from the surrounding fluid environment and form microcolonies. Biofilm reach their ultimate thickness >100mm.
Stage V: Dispersion	Bacterial cells are released from biofilm into the surrounding environment. The detached bacteria find new locations and restart the new biofilm formation. Detachment of biofilm aggregates by physical forces.

(Singh et.al. 2019)

Table.3

Biofilm forming genera in Dairy Industry	Why they form biofilm?
1. <i>Bacillus</i>	Present in raw and even pasteurized milk, Example – <i>B. subtilis</i> (It requires mainly carbon and energy to make the biofilm and use a number of sugars, organic acids and different organic compounds for this task.)
2. <i>Pseudomonas</i>	Example – <i>P. fluorescens</i> (It is well-known for this cause because of its high heat resistance and short generation time and these characteristics make it a successful biofilm former)
3. <i>Listeria</i>	Example- <i>Listeria monocytogenes</i> (It is mainly affected by temperature, strain origin and nutrient level and also has the property of attachment to surfaces passively and its biofilms are

	primarily comprised of teichoic acids which can grow on polypropylene, steel, rubber and/or glass surfaces)
4. <i>Staphylococcus</i>	Example- <i>Staphylococcus epidermidis</i> (In the process of staphylococcal biofilm formation, the accumulation and development of a mature stage depend mainly on the polysaccharide intercellular adhesions (PIA) that promote bacterial accumulation, especially polysaccharide poly-N-succinylb- 1-6 glucosamine (PNAG))
5. <i>Streptococcus</i>	Example- <i>Streptococcus. Thermophilus</i> (Affects mainly cheese and pasteurized milk – In the heating chamber of the section where temperature remains within 30 to 730C lies, the maximum degree of biofilm formation occurs. As a result the defects in milk and cheese quality like acidic flavour and undesirable texture are spotted.)
6. <i>Lactobacillus</i>	Example- <i>Lactobacillus</i> like <i>L. rhamnosus</i> (Biofilm formation by <i>Lactobacillus</i> spp. Is relatively beneficial because of its property of colonization and longer mucosal permanence of the host as these help in avoiding pathogenic bacterial colonization)
7. <i>E. coli</i>	Example- <i>E. coli</i> (The autoinducer- 2 (AI-2) of <i>E. coli</i> O157:H7 act as supplementary force for biofilm production as AI-2 signals regulate chemotaxis, flagellar synthesis and motility of genes. The <i>E. coli</i> O157:H7 yields exopolysaccharides (EPS) which helps in cell attachment and formation of 3D structures of biofilms.)

(Singh et.al., 2019)

Table.4 Foodborne outbreaks caused by cross-contamination involving work surfaces

Pathogen	Involved food	Contamination pathway
<i>Escherichia coli</i> O157:H7	Flavored yogurt	Pump used previously for unpasteurized milk
<i>Salmonella berta</i>	Soft cheese	Cheese ripening cubes previously used for chicken carcasses
<i>Salmonella enterica</i>	Ice cream	Ice cream transporting cistern previously used for raw egg
<i>Listeria monocytogenes</i>	Butter	Food plant environment
<i>Yersinia enterocolitica</i>	Pasteurized milk	Environmental contamination after processing

(González-Rivas et.al, 2018)

Table.5 Microorganisms involved in biofilm formation in different processes:

Microorganism	Dairy Process surface
<i>Bacillus species</i>	Ultrafiltration and reverse osmosis, membranes, evaporators
<i>E.coli</i>	Ultrafiltration, membranes
<i>Pseudomonas</i>	Storage tank, Ultrafiltration
<i>Acientobacter</i>	Milk transfer lines
<i>Streptococcus thermophilus</i>	Milk pasteurization & cheese manufacture

(Singh et.al., 2019)

Table.6 Overview of biofilm problem areas at dairy farms and dairy processing plants

Sampling points	Materials	<i>Pseudomonas</i>	<i>Staphylococcus</i>	<i>Bacillus</i>	LAB	<i>Enterobacteriaceae</i>	<i>Listeria</i>
Balance tank	Steel	-	-	+	-	+	-
Aging tank	Steel	-	+	-	-	-	-
Feeding unit	*	-	-	+	+	+	-
Conveyer belt of packaging machine	Rubber	+	-	-	+	+	-
Ultrafiltration membrane	Steel	-	-	-	+	-	-
Short milking tube	Rubber	+	+	-	+	+	-
Floor Drain	*	-	-	-	-	+	+

(Marchand et.al, 2012)

Table.7

Natural Ingredients	Description
1. Ginger extract	Major constituents in ginger rhizomes are Carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds. Effective against - Potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), digestive health (indigestion, constipation and ulcer), cardiovascular disorders (atherosclerosis and hypertension), vomiting, diabetes mellitus, and cancer. It also has anti-inflammatory and anti-oxidative properties for controlling the process of aging. Bacterial biofilm formation can cause serious problems in clinical and industrial settings, which drives the development or screening of biofilm inhibitors. Some biofilm inhibitors have been screened from natural products or modified from natural compounds. Ginger has been used as a medicinal herb to treat infectious diseases for thousands of years, which leads to the hypothesis that it may contain chemicals inhibiting biofilm formation.
2. Essential oils:	Used as natural preservatives and sanitizers in the Dairy Industry. Action - Essential oil damages the cell wall and membranes of microorganisms, alter the morphology and coagulate the cytoplasmatic material. Research reported the good antimicrobial effects of Essential oil on pathogenic and spoilage bacteria. Besides this, they have good anti-biofilm forming and anti-QS effect. The strong aroma of essential oil can affect organoleptic properties of foods. Essential oil which combines antimicrobial efficiency gives a pleasant flavour effect. These results lead to the conclusion that essential oils can be used as alternative sanitizers and preservatives in the food industry.
3. Berberine alkaloid- isolated	Effective against- Antimalarial, antisecretory, and anti-inflammatory as well as anticancer activities with relatively low cytotoxicity. It has also been reported that

<p>from various Chinese herbs, including <i>Hydrastis canadensis</i>, <i>Berberis aristata</i>, <i>Coptis chinensis</i>, <i>Coptis rhizome</i>, <i>Coptis japonica</i>,</p>	<p>berberine is useful in the treatment of gastroenteritis, diarrhea, and cholera diseases, Improve intestinal health and lower cholesterol Berberine has antimicrobial activity against several bacterial species, and interferes with the adherence of <i>Streptococcus pyogenes</i> to host cells, either by preventing the complexing of lipoteichoic acid with fibronectin or by dissolution of such complexes once they are formed.</p>
<p>4. Honey</p>	<p>Honey may have a similar antibacterial effect on <i>Streptococcus mutans</i>, which is considered the main causative organism of dental caries. <i>S. mutans</i> along with other oral bacteria forms on the tooth surface a microbial community surrounded by extracellular matrix and salivary proteins collectively known as dental biofilm.</p>

(Baheerati, 2016)

Table.8 Novel safe approaches for the control of biofilm formations

<p>1.Polysaccharides</p>	<p>Can inhibit the biofilm formation of bacteria, possibly by modifying the physical properties of both abiotic and biotic surfaces. It was shown that <i>E. coli</i> exopolysaccharides can alter the abiotic surface properties such as increase the hydrophobicity of glass surfaces and also can prevent cell-to cell auto aggregation via adhesions of bacteria</p>
<p>2.Enzymes</p>	<p>Serine proteases were efficiently reducing <i>Bacillus</i> biofilms whereas polysaccharides remove more efficiently <i>Pseudomonas fluorescens</i> than serine proteases. Polysaccharide polymerases and esterase can also control biofilm formations.</p>
<p>3.Nisin- produced by some strains of <i>Lactococcus lactis</i> and has been employed as an antibiofilm agent</p>	<p>Nisin has a mode of action that results in the formation of pores in the cell membrane of the bacteria. Pore formation leads to cell lysis and death. The bactericidal activity of nisin has been shown to target other Gram positive bacteria closely related to <i>Lactococcus lactis</i> and some Gram positive pathogens, such as <i>Listeria monocytogenes</i>. Nisin is effective against planktonic cells of multi-drug resistant staphylococci</p>
<p>4. Citric acid- alternative disinfectant in controlling biofilm formation in the dairy industry.</p>	<p>The prevention and removal of biofilm formation of <i>S. aureus</i> strains isolated from raw milk by citric acid treatments (2% and 10%) for 20 min were assessed for comparison with peracetic acid treatment (0.3%) on both on microtitration plate and stainless steel coupons. The prevention and removal of biofilm formation ratios and the numbers of prevented or removed <i>S. aureus</i> strains were observed to be higher by using citric acid treatments compared with peracetic acid treatment on both surfaces. Moreover, the prevention and removal of biofilm formation were substantially higher when the concentration of citric acid treatment increased from 2% to 10% and the stainless coupons were used.</p>
<p>5.Gallic acid- phenolic products found in plants</p>	<p>It has been shown that gallic acid has strong antimicrobial activity against several bacterial strain also reported antibiofilm activity of</p>

such as tea leaves, fruits and flowers	gallic acid for the prevention and removal of <i>E.coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> and <i>L.monocytogenes</i> biofilms. The researchers found that gallic acid can prevent and remove these pathogens by promoting reductions in biofilm activity >70% of all tested microorganisms.
6.Malic acid	The antimicrobial action of malic acid is to lower the pH value or cause the significant damage to the cytoplasm of bacteria also found that malic acid was also effective in food industry for complete inhibition of <i>Salmonella typhimurium</i> biofilm in carrot and other food contact surfaces.

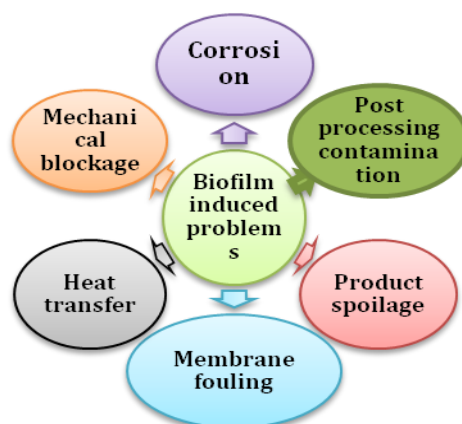
(Meltem, 2015)

Table.9 Different natural elemental extracts effective against various microbial biofilms

Element(s)	Extract	Against
<i>Epimedium brevicornum</i>	Plant extract	<i>Propionibacterium acne</i>
<i>Malus pumila</i>		
<i>Polygonum cuspidatum</i>		
<i>Rhodiola crenulata</i>		
<i>Dolichos lablab</i>		
<i>Melia dubia</i>	Bark extract	<i>E. coli</i>
<i>Capparis spinosa</i>	Plant extract	<i>E. coli</i> , <i>Pseudomonas aeruginosa</i>
Cinnamon	Cinnamon oil	<i>S. mutans</i> & <i>Lactobacillus plantarum</i>
Cinnamon	Cinnamon oil	<i>Staphylococcus epidermidis</i>
<i>Cinnamomum cassia</i>	EO	Enteropathogenic <i>E. coli</i> & <i>L. monocytogenes</i>
Oregano	Oregano EO	<i>Staphylococcus</i> & <i>E. coli</i>

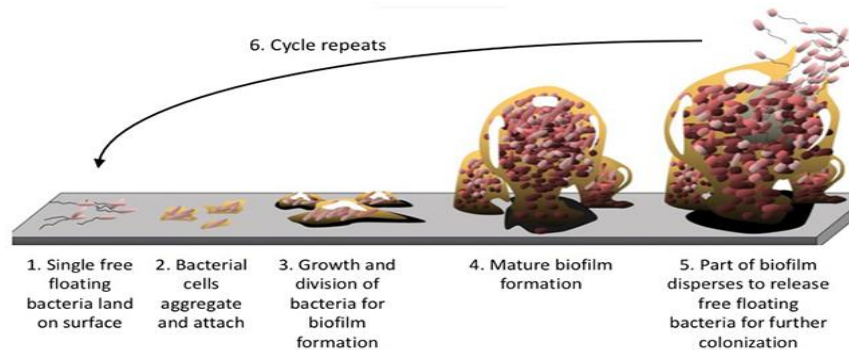
(Singh et.al. 2019)

Fig.1 Biofilm induced problems



(Srey et al., 2013)

Fig.2 Biofilm formation process



Biofilm forming microbes of dairy industry

Microorganisms occurring in the food industry could be a source of secondary contamination in food products. The other important biofilm forming genera of dairy industry are *Bacillus*, *Pseudomonas*, *Listeria*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *Salmonella typhimurium* and *Coronobacter sakazakii* etc.

Cross-contamination in the food industry is defined as direct or indirect microbial transference from a contaminated to a noncontaminated matrix, which can be food, work surfaces, or workers, among others.

Natural ingredients against biofilm formation

Biofilm formation of pathogenic bacteria on surfaces and on foods represents a big challenge to the food industry and healthcare. The removal of biofilms is difficult and in spite of the efforts for good sanitization surfaces and products can be contaminated which can lead to severe health problems so Biofilm formation can be reduced by various natural ingredients.

In conclusions, this biofilm formation possesses profound implications and throws a major challenge to the dairy sector where they

act as the principal reservoir of microbial contamination. These lead to financial crisis by impairment of raw material and its products. So it is important to understand the biofilm formation process, different biofilm formation genera in dairy industry and their occurrence on different surfaces etc. Biofilm formation can be reduced by various natural ingredients. Biofilm causes direct or indirect impact on food product contamination so it is one of the alarming niche in dairy industry. It is emphasized that good manufacturing practice, good hygienic practice and hazard analysis and critical control point should be implement in dairy industry to prevent the contamination of dairy products.

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