Importance of Biofilms on Dairy Industry Safety

Dilvin İpek*,[1] and **Nükhet N. Demirel Zorba**[2]

[1] Department of Food Processing, Ezine Vocational School, Çanakkale Onsekiz Mart University, 17600 Ezine, Çanakkale, Turkey
[2] Department of Food Engineering, Çanakkale Onsekiz Mart University, 17020 Çanakkale, Turkey

Microbial adhesion and biofilm formation on process surfaces and also their cross-contamination to dairy products are important food safety problems in dairy industry which has a high consumption ratio in the world markets. Raw materials, process lines and other surfaces in production area and packaging materials are the points that biofilm can formed and contaminate the final product. *Staphylococcus*, *Streptococcus*, *Bacillus*, *Pseudomonas* spp., Enterobacteriacea, *Debaryomyces* and *Saccharomyces* spp. etc. can form biofilm on dairy process line and process surfaces (wall, drainage, door and floor). This structure can form corrosion on surfaces and also increase the energy usage, hygiene and sanitation, treatment costs, final product loss, hazards for consumer health. Since the realizing of this issue importance, researchers have tried to understand biofilm forming microorganisms, their hazards in dairy industry and investigate right cleaning strategies. This review aims to show importance of biofilms on dairy industry and food safety as well as discusses to emerging controlling and preventing strategies.

Keywords: Biofilm, food safety, dairy industry, human health

1. Introduction

Dairy products have a big importance for human nutrition and many kinds of dairy products are consumed. So, dairy industry has a big part in world markets [1, 2]. Spoilage and deterioration could cause big problems about economy and consumer health. Food safety is an issue that should be given importance.
Attachment and micro-ecosystem formation of microorganisms on surfaces are named as biofilms. Biofilms could be formed in pipelines, instrument surfaces and on the all production area (doors, walls, floor, windows etc.). Exopolysaccharide biofilm structure protects to microorganisms from chemical cleaning agents and helps to symbiotic life. Microorganisms can survive as heterogenic cultures and exchange genetic materials in biofilms. Resistant-microorganisms can easily occur on process lines and surfaces. Inadequate cleaning process is also helps to biofilm forming.

Biofilm formation is a key point for food safety since it could change and also increase probability and frequency of the determined microbiological hazards. New risks and/or critical control points could be added to risk analyses of the product depending biofilm formation. It is one of the source for infections and outbreaks like Listeriosis and Salmonellosis. 80% of bacterial infections were found that connected with biofilms in USA [3]
Microorganisms can naturally attach and form biofilms on the wet and nutritive surfaces. They produce exopolysaccharides and form to biofilm matrix on dairy industry process lines and surfaces [2].
Currently in dairy industry, chemical based cleaning procedures are used which are named clean-in-place (CIP) and clean-out of-place (COP). Therefore, there are many reports about chemical resistance bacteria in dairy industry's surfaces, products and wastewater systems. Biofilm structure also helps to bacteria for gaining resistance [4, 5]
The aim of this study is to show importance of biofilms on dairy industry and food safety as well as discusses to emerging controlling and preventing strategies.

2. Biofilm Formable Microorganisms' Cases in Dairy Industry

Bound to the nutrient value of raw material, moisture of dairy products and humidity of process area/surfaces; attachment and biofilm formation ratios of microorganisms should be increased. Microorganisms like Enterobacteriacea, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Lactococcus*, *Listeria*, *Streptococcus*, *Staphylococcus*, *Debaryomyces*, *Saccharomyces* and *Raoultella* sp. were isolated from dairy process lines, production surfaces and wastewaters [2, 5]. Isolated bacteria was given in Table 1.
Sharma and Anand [6] isolated 36 pure isolates which were identified as *Bacillus cereus*, *Esherichia coli*, *Staphylococcus aureus*, *Shigella* spp. from pilot type milk process lines. Temelli et.al. [1] studied on a Turkish white cheese process lines and area which include process lines, air, walls and doors. They determined *Staphylococcu*s spp., psychrophilic bacteria, yeast and mold from 30 sample points (.raw milk, pasteurized milk, milk in cheese vat, curd, moulded cheese before salting, moulded cheese after salting, cheese at cold holding and vacuum packaged cheese; samples from starter culture, rennet, calcium chloride solution, brine, cheese vat, cheese cloth, polyethylene separator sheet, milk stirrer, curd cutting knife, side pressure plate, upper pressure plate, moulded cheese cutting knife, cheese tray, packaging material used during production; workers_ hands, cold room and production room air, floor, wall, and potable water). İpek and Zorba [7] studied on Old Kashar cheese which is very similar to Gouda cheese and Gruyere in 2009. They choose sample points

according to a microbial problem (pink dots). Sampling points were chosen as water, salt, moulding equipments, air, pasteurization tank, maturation shelves. Researcher isolated and identified E*dwardsiella* spp., *Klebsiella* spp., *Debaryomyces* spp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Cladosporium* spp. from Old Kashar cheese's process lines. They examined that the main contamination surface was maturation shelves. Gunduz and Tuncel [8] isolated Enterobacteriaceae, *Aeromonas*, *Plesiomonas*, *Moraxella*, *Pseudomonas*, *Alcaligenes*, *Streptococcus*, *Lactococcus*, Citrobacter, *Proteus* spp. *Listeria monocytogene*s and *Shigella* spp. from ice cream process lines and area. Samples were taken by using stainless steel coupons (pasteurizer, balance tank, aging tank, feeding unit, conveyor belt, floor drain and doormat) .They determined that all these bacteria were biofilm formable. Ölmez [9] isolated *Listeria* spp., Coliform bacteria, *Lactococcus*, Lactobacillus and Staphylococcus spp. biofilm formable bacteria from UHT milk process lines before and after cleaning procedures. They choose their sampling points by following CIP cleaning procedures. İpek and Zorba [2] studied on a traditional semi-hard and maturated white cheese "Ezine Cheese" process lines. Ezine cheese production has a batch type. So, CIP and COP procedures were used for cleaning. According to these prosedures, sampling points were chosen (water, air, raw milk tank, balance tank, plastic pipes, stainless steel pipes, cheese vats, cheese cloth, knieves, curd cutting knieves, wall, drainage, production and filling personnel, brine tank, plastic cups). Researcher isolated generally *Listeria*, *Pseudomonas*, *Ochrobacterium*, *Mannheimia*, *Klebsiella*, *Enterobacter*, *Bacillus*, *Geobacillus* and *Streptococcus* spp. from Ezine cheese process lines and process area which included air, walls, drainage and water. They reported that *Bacillus* sp. was the most common isolated from surfaces and biofilm formation capacity of Enterobacteriacea members were very high. Drainage, air and water entries were determined as important contamination points. Dixon et.al. [5] studied about biofilm potential of primary treated dairy wastewater. Isolates were taken from a dairy milk powder plants' primary treated wastewater system. In this study, *Pseudomonas*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Raoultella* spp. and *Bacillus cereus* were isolated from dairy wastewater. Six of these microorganisms along with *Raoultella* spp. were determined strong biofilm former.

Chemical resistance microorganisms are very common and important for dairy industry. These type microorganisms can be isolated from dairy process lines, area and wastewater systems. As an important point, this type microorganisms can communicate each other for growing, forming biofilm and also transfer their resistant genes to each other [4, 5]. Ryu and Beucat [10] reported that *Esherichia coli* O157:H7 strains can form biofilm on stainless steel 304 (which is very common surfaces in dairy industry) and also, this strains became resistance to chlorine after cleaning treatments like dairy industry. Gadea et.al. [11] were reported benzalkonium chloride, hexadecylpyridinium chloride and some antibiotics (amplicilin, cefotaxime, imipenem, tetracycline etc.) adaptable *Bacillus* sp., *Staphylococcus* sp and Enterobacteriacea from 39 organic foods which includes flours, fruits and vegetables, legumes, cereals, rice, pastes, sauces, cheeses and manufactured products. As a PhD thesis, İpek [12] were examined to regional white cheese producers' process lines and process area surfaces. During the study; chlorine, perasetic acid and 70 % alcohol resistance *Bacillus* sp., *Pseudomonas* sp., *Listeria* sp., Enterobacteriacea were reported from dairy process lines (like tanks, vats and pipes), drainage and water. They reported that inadequate hygiene and sanitation procedures, and also biofilm structure help microorganims to gain chemical resistance. Tepeli and Zorba [13] studied about extended spectrum β-lactamase (ESBL) and AmpC β-lactamase activities of Enterobacteriaceae isolated from raw milk and cheese production line. They reported β-lactamase (ESBL) and AmpC β-lactamase active Enterobacteriacea isolates which were generally isolated from bulk milk tanks. Also they reported that effective food safety and hygiene practices should be performed to reduce cross-contaminations in dairy plants.

Table 1. Isolated bacteria from dairy industry

Isolated Place	Bacteria	References
Milk-Process lines	*Bacillus cereus*, *Esherichia coli*, *Staphylococcus aureus*, *Shigella* spp.	Sharma and Anand [6]
Turkish White Cheese Process Line	*Staphylococcus* spp., psychrophilic bacteria, yeast and mold	Temelli et.al.[1]
Old Kashar process line	*Edwardsiella* spp., *Klebsiella* spp., *Debaryomyces* spp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Cladosporium* spp.	İpek and Zorba [7]
Ice Cream process lines	*Listeria monocytogenes*, *Shigella* spp.	Gündüz and Tuncel [8]
Milk process lines	*Listeria* spp., Coliform bacteria*, Lactococcus* spp., *Lactobacillus* spp., *Staphylococcus* spp.	Ölmez [9]
Ezine cheese process lines (a kind of Turkish White Cheese)	*Listeria* spp., *Pseudomonas* spp., *Ochrobacterium* spp., *Mannheimia* spp., *Klebsiella* spp., *Enterobacter* spp., *Bacillus* spp., *Geobacillus* spp., *Streptococcus* spp.	İpek and Zorba [2]
Primary treated dairy wastewater	*Pseudomonas*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Raoultella* spp. and *Bacillus cereus*	Dixon et.al. [5]
Stainless steel 304 (which is very common surfaces in dairy industry)	Chlorine resistance *Esherichia coli* O157:H7	Ryu and Beucat [10]
39 organic foods which includes flours, fruits and vegetables, legumes, cereals, rice,	Benzalkonium chloride, hexadecylpyridinium chloride and some antibiotics (amplicilin, cefotaxime, imipenem, tetracycline etc.)	Gadea et.al. [11]

pastes, sauces, cheeses and manufactured products.	adaptable *Bacillus* spp., *Staphylococcus* spp and Enterobacteriacea	
Dairy process lines (like tanks, vats and pipes), drainage and water.	Chlorine, perasetic acid and 70 % alcohol resistance *Bacillus* spp., *Pseudomonas* spp., *Listeria* spp., Enterobacteriacea	İpek [12]
Dairy process lines (especially bulk milk tanks)	β-lactamase (ESBL) and AmpC β-lactamase active Enterobacteriacea	Tepeli and Zorba [13]

3. Monitoring the Biofilm Structure

Biofilm formation is a key point for food safety since it could change and also increase probability and frequency of the determined microbiological hazards. New risks and/or critical control points could be added to risk analyses of the product depending biofilm formation. So monitoring the biofilm structure is very important to understanding and developing new control and/or prevention strategies.

Congo red agar method is used for determining the biofilm forming features of microorganisms. This method was developed by De la Fuente et al. [14] is based on the subculture of the bacterial strains on brain heart infusion agar with sucrose and Congo red dye. Pigmentation on this special medium gives us information about microorganism's biofilm forming capacity. Biofilm formable microorganisms EPS and medium materials react and pigmentation occurs. If the pigment is black, the microorganism is named as biofilm positive [15, 16]. Microplate method is the most used method, the standard method for determining the biofilm formation. In this method 96 well microplates, crystal violet (as indicator) and Elisa reader or spectrophometer are used. According to OD values, researchers can evaluate the bacterial adhesion and biofilm formation. In this method 24 h microorganisms are used and reading is done at 640 nm. According to calibration curve of OD640nm versus cfu/ml, biofilm formable bacteria on microplate surfaces can be detected [17, 18].

Flow cytometry is an excellent tool for studies of biofilm physiology. Cell enumeration, cell viability, membrane integrity, metabolic activity and cell sorting can be learned or determined by using flow cytometry. Also activity ratio of microorganisms can be determined. This technique evaluates attached surface cells of biofilms one by one from prepared homogenous biofilm suspension. Williams et. al. [19] determined the physiological changes of *Staphylococcus aureus* by using this techniques during biofilm formation on a surface. Also this method can be useful for determining antimicrobial agent activity on microorganisms [20, 17]. In pipe line systems, this method also gives high speed monitoring chance for microorganisms. Besides biofilm formable microorganisms which cannot detected easily by using standard methods can be determined and isolated by using this method. By using flow-cytometer, Harnisch et. al. [21] determined *Geobacter sulfurreducens* which cannot detected even with molecular methods.

Fluorescence and confocal laser scanning microscopy are used for determination of the biofilm formed microorganisms' structures or viability. By using Baclight Live/Dead (1:1 propidium iodide-PI and SYT09) to stain for bacterial viability and DAPI (4,6-diamino-2-pheynylindole) for total biofilm cell analysis. In Baclight Live/Dead method; bacteria with intact cell membrane stain fluorescent green, whereas bacteria with damaged membranes strain fluorescent red. By using confocal scanning microscopy, we can have 3-D projection of biofilm structure. Fluorescence in situ hybridization (FISH) method is a method for detection of mixed populations by using fluorescence microscopy. In this method a nucleic acid probes which focus on 16S/18S rRNA sequences [17, 22, 23].

Protein analyses is another method for determining biofilm-immobilized microorganisms. Free and immobilized cells can be obtained one or two dimensional electrophoresis and its differences can identified by mass-spectrometry (MS) or liquid chromatography-mass spectrometry (LC-MS) based methods. Biofilm and planktonic cells of same microorganisms can be compared by this method. Also identification and characterization of protein samples are done [17]. Diethmaier et. al. [24] determined the YmdB proteins on *Bacillus subtilis* biofilm formation behaviour. They determined that damage of YmdB proteins producer genes affects the biofilm formation negatively.

Molecular methods are popular methods for biofilm determinations. Quantitative PCR (q polymerase chain reaction) is the most used techniques. These techniques help to the amplifications and simultaneous quantification of target DNA molecules by using fluorescent dyes. This method is golden standard techniques also. It is a RNA based method, so RNA quality and purity are very important. Also this method reflects to genes which are being actively expressed with in a biofilm. The Ibis technique is a novel method for bacterial detection based on species-specific nucleotide composition. This technique has specially designed polymerase chain reaction (PCR) primers for amplifying to target specific nucleotide. Then mass of the PCR-amplified product is measured by mass spectrometry and referenced to a database for identification of the particular bacterial species. The detection time is relatively short (<6 hours) and this technique is applicable to biofilms [17, 25].

Light scattering techniques such as low-coherence interferometry (LCI) and optical coherence tomography (OCT) are used for detecting biofilm infections. These methods are non-invasive methods which can evaluate the marked differences in optical scattering generated by biofilm infections versus non-infected tissue and can be modified and applied in support of biofilm detection in the food industry [26, 27]. Pavlovsky et. al. [27] determined the temperature effect on *Staphylococcus epidermidis* biofilms by using light scattering techniques. By using these techniques; morphology, cell viability, the polymeric properties of the extracellular polymeric substance (EPS), and the rheological properties of the

bulk biofilm could be determined. According to results; cell viability and biofilm morphology decreased by using heat treatment (45ºC and 60ºC), but EPS structure of biofilm was not significantly degraded.

Raman and infrared spectroscopy are complementary vibrational spectroscopy techniques which can give biochemical information of biofilms. This information can be provided nearly one hour. These methods can also detect to "viable but non-culturable bacteria" [29]. Raman technology is an effective technology for observing mono and multi species. However, Ivleva et.al. [30] studied about in situ surface-enhanced raman scattering analysis of biofilm and this techniques was determine the biofilm structure and chemistry 2 times more than Raman technology.

Hyperspectral imaging devices are used for determination biofilm on surfaces (e.g. stainless steel, granite, plastic). Some microorganisms' biofilms (such as *E. coli* O157:H7 and *Salmonella* sp.) can be detected on surfaces by using their characteristic fluorescence emission in ultraviolet-A light. It is a hand-held device that detects three wavelengths has been developed for inspecting the efficacy of sanitisation procedures [19, 31].

Piezoelectric is electromechanical biosensors for early biofilm detection. This sensor is put in surfaces. When bacteria accumulate on sensor's surface, its impedance changes and early biofilm formation can be detected [31]. Gula et.al. [32] researched about Piezoelectric tuning fork based mass measurement method as a novel tool for determination of antibiotic activity on bacterial biofilm. By this techniques, Tuning forks resonance frequency (68 Hz and 24 Hz) was used on *Pseudomonas aeruginosa* biofilm. Piezoelectric technique for antibiotic activity (gentamicin and ciprofloxacin) was observed in correlation with microplate assay.

Chip calorimeter is a new monitoring method for real-time information about the physiological state of biofilms. This method uses metabolic activity heat measurement for real time results. It can be used for determining the effects of antibiotics and other biocides. Growth inhibition and inactivation effects of inhibitors on biofilm bacteria were quantified by analysing the metabolic heat production rate. As a result, a concentration dependent manner of growth inhibition and inactivation was found demonstrating the suitability and sensitivity of the methodology [33, 34]. Buchholz et.al [33] use this method for determining a hunter bacteria (*Bdellovibrio bacteriovorus*) effects on Pseudomonas spp. biofilms. Heat signal gives information about biofilm activity. Biovolume versus heat production rate graphic was drawn and according to this graph revealed to *Pseudomonas* spp. biofilm statues. The results correlated to confocal laser scanning microscope (CSLM) results.

ATP-bioluminance methods are based on the reaction between luciferin enzyme and the oxygen. When this reaction takes place, light output occurs. Light output, and ATP content, is measured in relative light units (RLU) by using luminometer. Microbial ATP and also organic material ATP are measured together in this method. Microbial load of surfaces are measured a little bit more than real microbial load. So, sanitation of production line and personnel hygiene can be provided before formation of high microbiological contamination with this method [35, 2].

4. Emerging Control and Prevention Strategies

Biological and chemical based control method are used for Biofilm formation control and prevention in dairy and food industry.

4.1 Biological Based Control and Prevention Strategies

Bacteriophages are viruses which are specific for their host bacteria. Hunter bacteria is generally gram negative, small type bacteria that needs their prey for its life circle. According to this features, bacteriophages and hunter bacteria can be used as control method for biofilm forming. Microorganisms can reach to target bacteria in mono or mixed-cultured biofilm structures and marked and/or destroyed the target bacteria cells. As an important point, this method can help to formation of phage-resistant bacteria and biofilm structure [17]. Sillankorva [36] determined the Phage φIBB-PF7A and φIBB-SL58B on *Pseudomonas fluorescens* and *Staphylococcus lentus*. As a result of this study, Phage φIBB-PF7A was found more effective on the host-bacteria biofilm structures. Gheorghe et al. [37] studied about three-phage cocktails effects on *Listeria monocytogenes* biofilms on stainless steel and they determined an important decrease. Montañez-Izquierdo et. al. [38] determined nearly same result on *Listeria monocytogenes* biofilms on stainless steel by using P100 phage. Zhang and Hu [39] determined effectiveness of phage therapy on *Pseudomonas aeruginosa* biofilm samples from waste water systems. Hunter bacteria method that use predator-prey interactions is non-invasive, fast and non-destructive and applicable to nearly all kind of microorganisms. As an example *Bdellovibrio bacteriovorus*; *Bdellovibrio* spp. is gram negative, small and fast moving bacteria without flagella as a hunter of other gram negative bacteria. These bacteria live like bacteriophages, discovered and characterized. Also they are use as predator for *Pseudomonas* spp. biofilm. According to this specific hunter bacteria effects on bacteria, differences of specific target bacteria can be measured. Buchholz et.al. [33] used *Bdellovibrio bacteriovorus* on *Pseudomonas* spp. biofilm in their study. They observed metabolic heat production activity of the biofilm and hunter bacteria effect on target biofilm-formed bacteria.

Antagonism can be defined as negative effects of some microorganisms on other microorganisms' growth. In biofilm structure, mono or mixed-culture microorganisms can survive with symbiotic effect. This method can be used for mono cultured biofilms and also based microorganisms of mixed-culture biofilms. In lots of researches; *Lactobacillus* spp. are used for this effect, because this species has strong antagonistic effect with their bacteriocins [37, 40]. Huang et.al. [41]

determined Bacillus TKS1-1, OF3-16, SP4-17, HSP1, WG6-14, TLB7-7, and WP8-12 strains antagonistic effects on *Xanthomonas axonopodis pv. citri* which can cause citrus bacterial canker disease resulting in significant crop losses. These strains use as biocontrol agents and found effective for this disease. De la Fuente et.al. [14] studied about antagonistic effect of *Pseudomonas fluoresces* FF48 on *Flavobacterium psychrophilum* biofilm structure. They determined that *Pseudomonas fluoresces* FF48 produce siderophore and this chemical has an antagonistic effect on fish pathogenic species *F. psychrophilum*.

4.2 Chemical Based Control and Prevention Strategies

Quorum sensing is a chemical based communication method between microorganisms. QS Involve a density-dependent recognition of signalling molecules (auto inducers, AI), resulting in modulation of gene expression. Biofilm formation on a surface depends on the production of various signaling molecules like N-acyl homoserine lactones (AHLs). As biofilms typically contain high concentration of cells, AI activity and QS regulation of gene expression have been proposed as essential components of biofilm physiology. It is believed that quorum sensing inhibition may represent a natural, widespread, antibiofilm strategy. Quorum quenching is interrupting this communication by using enzymes, chemicals, natural extracts and antimicrobial agents and can inhibit to biofilm formation [12, 18, 42].

Nanotechnology is using for biofilm formation control nowadays. Nanoparticules of metals (silver, TiO2), chemicals and antimicrobial agents are used for this technology. Nanotechnological surfaces, coatings, packages, disinfectants and aerosols have been researched. There are lots of study about nanoparticles success on biofilm control [43, 44]. However, there are not enough study about this subject in the literature. Zhang et. al. [45] studied about urease gold nanoparticles composite nanomaterial on an egg shell biofilms and urease AuNPs composite nanomaterial was determined 7-fold higher effective on biofilms. Manjumeena et.al. [46] studied on antibacterial and antifungal effect of AgNPs-coated Reverse osmosis (RO) membranes on *Esherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* and fungal strains such as *Candida tropicalis*, *C. krusei*, *C. glabrata*, and *C. albicans*. According to result of this study, coating was determined more effective on bacteria. However, coating was less effective on *Pseudomonas aeruginosa*.

5. Conclusion

In dairy industry, safety and good manufacturing practices are very important for the sustainability, economy and consumer health. However, biofilms can easily form as microbial hazards on dairy process lines (tanks, heat exchangers, pipes, small vehicles etc.), process area (walls, doors, floors, drainages, air conditions) surfaces and process water. This structure can injure the surfaces and attach permanently that can be a reason of the cross-contaminations. Pathogen and also chemical resistant bacteria can be detected in this surface structure. This review aims to show importance of biofilms on dairy industry and food safety as well as discusses to emerging controlling and preventing strategies. As a result; understanding, monitoring and controlling/prevention of this structure is very important not only for dairy industry but also for food industry and significantly important for consumer health.

References

[1] Temelli S, Anar Ş, Sen C, Akyuva P. Determination of microbiological contamination sources during Turkish white cheese production. Food Control. 2006; 17(11), 856-861.
[2] İpek D, Zorba N N D. Microbial load of white cheese process lines after CIP and COP: A case study in Turkey. LWT. 2018; 90, 505-512.
[3] Sprey S, Jahid F K, Ha S. Biofilm Formation in Food Industries: A Food Safety Concern. Food Control. 2013; 31:572-585.
[4] Tezel U, Pavlostathis S G. Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. Current opinion in biotechnology. 2015; 33, 296-304.
[5] Dixon M, Flint S, Palmer J, Love R, Biggs P, Beuger A. Analysis of culturable and non-culturable bacteria and their potential to form biofilms in a primary treated dairy wastewater system. Environmental technology. 2018; 39(17), 2185-2192.
[6] Sharma M, Anand S K. Biofilms evaluation as an essential component of HACCP for food/dairy processing industry–a case. Food control. 2002; 13(6-7), 469-477.
[7] İpek D, Demirel Zorba N N, editors. Kaşar Peyniri Üretim Hattı Microflorasının Belirlenmesi (Determination the Old Kashar Cheese Process Lines' Microflora). Türkiye 3. Ulusal Veteriner Gıda Hijyeni Kongresi (3 th National Veterinary Food Hygiene Congress); May 14-16; Bursa, Turkey. 2009.
[8] Gündüz GT, Tuncel G.. Biofilm formation in An Ice Cream Plant. Antonie van Leeuwenhoek. 2006; 89 (3): 329-336.
[9] Ölmez Z. Süt Sanayisinde Biyofilm Oluşturan Mikroorganizmalar ve Biyofilm Oluşumunun Önlenmesi. Süleyman Demirel University. MsC Thesis., 2009. Turkey.
[10] Ryu J H, Beuchat L R. Biofilm formation by Escherichia coli O157: H7 on stainless steel: effect of exopolysaccharide and curli production on its resistance to chlorine. Applied and Environmental Microbiology. 2005; 71(1), 247-254.

[11] Gadea R, Fuentes M Á F, Pulido R P, Gálvez A, Ortega E. Effects of exposure to quaternary-ammonium-based biocides on antimicrobial susceptibility and tolerance to physical stresses in bacteria from organic foods. Food microbiology. 2017; 63, 58-71.
[12] İpek D. Applıcability of Ezine Cheese Process Lines Disinfection By New Disinfectant Formulas: Antibiofilm-Antiquorum Sensing Agents. PhD Thesis; 2017. p .166.
[13] Tepeli S Ö, Zorba N N D. Frequency of extended-spectrum β-lactamase (ESBL)–and AmpC β-lactamase–producing Enterobacteriaceae in a cheese production process. Journal of dairy science. 2018; 101(4), 2906-2914.
[14] De la Fuente M, Vidal J M, Miranda C D, González, G, Urrutia H. Inhibition of *Flavobacterium psychrophilum* biofilm formation using a biofilm of the antagonist *Pseudomonas fluorescens* FF48. SpringerPlus [online serial]. 2013; 2: 176.
[15] Arciola C R, Campoccia D, Gamberini S, Cervellati M, Donati E, Montanaro L. Detection of slime production by means of an optimised Congo red agar plate test based on a colourimetric scale in Staphylococcus epidermidis clinical isolates genotyped for ica locus. Biomaterials. 2002; 23(21):4233-4239.
[16] Gündoğan N, Ataol Ö. Et örneklerinden İzole Edilen Staphylococcus aureus ve Koagülaz negatif Stafilokok'ların Biyofilm Üretimi ve DNaz Aktivitelerinin Belirlenmesi. Türk Hijyen ve Deneysel Biyoloji Dergisi. 2012; 69(3):135-142.
[17] Azeredo J, Cerca N, Henriques M, Periera M O. Advanced Techniques in Biofilms. Braga: University of Minho Centre of Biological Engineering; 2014. ISBN: 978-989-97478-3-8.
[18] Kerekes E B, Deak E, Tako M, Tserennadmid R, Petkovits T, Vagvölgyi C, Krisch J.. Anti-biofilm forming and anti-quorum sensing activity of selected essential oils and their main components on food-related micro-organisms. J. Of Applied Microbiology. 2013; 115: 933-942.
[19] Williams I, Paul F, Lloyd D, Jepras R, Critchley I, Newman M, Warrack J, Grokarini T, Hayes A J, Renderson P F, Venables W A. Flow cytometry and other techniques show that Staphylococcus aureus undergoes significant physiological changes in the early stages of surface-attached culture. Microbiology. 1999; 145(6), 1325-1333.
[20] Barney J B, Clark D N. Resource-based theory: Creating and sustaining competitive advantage. Oxford: Oxford University Press; 2007.
[21] Harnisch F, Koch C, Patil S A, Hübschmann T, Müller S, Schröder U. Revealing the electrochemically driven selection in natural community derived microbial biofilms using flow-cytometry. Energy & Environmental Science. 2011; 4(4): 1265-1267.
[22] Saby S, Sibille I, Mathieu L, Paquin J L, Block J C. Influence of water chlorination on the counting of bacteria with DAPI (4', 6-diamidino-2-phenylindole). Applied and Environmental Microbiology. 1997; 63(4): 1564-1569.
[23] Lisle J T, Hamilton M A, Willse A R, McFeters G A. Comparison of fluorescence microscopy and solid-phase cytometry methods for counting bacteria in water. Applied and environmental microbiology. 2004; 70(9): 5343-5348.
[24] Diethmaier C, Pietack N, Gunka K, Wrede C, Lehnik-Habrink M, Herzberg C, Hübner S, Stülke J. A novel factor controlling bistability in Bacillus subtilis: the YmdB protein affects flagellin expression and biofilm formation. Journal of bacteriology. 2011; 193(21): 5997-6007.
[25] Costerton J W, Post J C, Ehrlich G D, Hu F Z, Kreft R, Nistico L, Kathju S, Stoodley P, Hall-Stoodley L, Maale G, James G, Sotereanas N, Demeo P. New methods for the detection of orthopedic and other biofilm infections. FEMS Immunology & Medical Microbiology. 2011; 61(2): 133-140.
[26] Nguyen C T, Jung W, Kim J, Chaney E J, Novak M, Stewart C N, Boppart S A. Noninvasive in vivo optical detection of biofilm in the human middle ear. Proceedings of the National Academy of Sciences. 2012; 109(24): 9529-9534.
[27] Tan S Y E, Chew S C, Tan S Y Y, Givskov M., Yang L. Emerging frontiers in detection and control of bacterial biofilms. Current opinion in biotechnology. 2014; 26: 1-6.
[28] Pavlovsky L, Sturtevant R A, Younger J G, Solomon M J. Effects of temperature on the morphological, polymeric, and mechanical properties of Staphylococcus epidermidis bacterial biofilms. Langmuir. 2015; 31(6): 2036-2042.
[29] Lu X, Al-Qadiri H M, Lin M, Rasco B A. Application of mid-infrared and Raman spectroscopy to the study of bacteria. Food and Bioprocess Technology. 2011; 4(6): 919-935.
[30] Ivleva N P, Wagner M, Horn H, Niessner R, Haisch C. In situ surface-enhanced Raman scattering analysis of biofilm. Analytical chemistry. 2008; 80(22): 8538-8544.
[31] Tang K, Zhang Y, Yu M, Shi X, Coenye T, Bossier P, Zhang X H. Evaluation of a new high-throughput method for identifying quorum quenching bacteria. Scientific reports. 2013; 3, 2935.
[32] Gula G, Waszczuk K, Olszak T, Majewska J, Sarowska J, Gotszalk T, Gutowicz J, Drulis-Kawa Z. Piezoelectric tuning fork based mass measurement method as a novel tool for determination of antibiotic activity on bacterial biofilm. Sensors and Actuators B: Chemical. 2012; 175: 34-39.
[33] Buchholz F., Wolf A, Lerchner J, Mertens F, Harms H, Maskow T. Chip calorimetry for fast and reliable evaluation of bactericidal and bacteriostatic treatments of biofilms. Antimicrobial Agents and Chemotherapy. 2010; 54(1): 312-319,
[34] Buchholz F, Lerchner J, Mariana F, Kuhlicke U, Neu T R, Harms H, Maskow T. Chip-calorimetry provides real time insights into the inactivation of biofilms by predatory bacteria. Biofouling. 2012; 28(3):351-362.
[35] Aycicek H, Oguz U, Karci K. Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. International journal of hygiene and environmental health. 2006; 209(2):203-206.
[36] Sillankorva S. Use of bacteriophages to control biofilms. PhD Thesis. University of Minho and University of Oulu. Braga. 2009, 170p.
[37] Gheorghe A, Jecu L., Voicu A, Popea F. Biological Control of Phytopathogen Microorganisms with Antagonist Bacteria [Internet]. [cited 2015 Sept 6]. Available from: http://www.aidic.it/IBIC2008/ webpapers /64Gheorghe.pdf.
[38] Montañez-Izquierdo V Y, Salas-Vázquez D I, Rodríguez-Jerez J J. Use of epifluorescence microscopy to assess the effectiveness of phage P100 in controlling Listeria monocytogenes biofilms on stainless steel surfaces. Food Control. 2012; 23(2): 470-477.
[39] Zhang Y, Hu Z. Combined treatment of Pseudomonas aeruginosa biofilms with bacteriophages and chlorine. Biotechnology and bioengineering. 2013; 110(1): 286-295.

[40] Olanrewaju O. Antagonistic effect of Lactobacillus isolates from Kunnu and Cowmilk on selected pathogenic microorganisms. Internet Journal of food safety. 2007; 9: 63-66.
[41] Huang T P, Tzeng D D S, Wong A C, Chen C H, Lu K M, Lee Y H, Tzeng K C. DNA polymorphisms and biocontrol of Bacillus antagonistic to citrus bacterial canker with indication of the interference of phyllosphere biofilms. PLoS One. 2012; 7(7), e42124.
[42] Kala, R., Chauhan, H., Rajput, A. and Kutty, R. 2012. Biofilm Characterirization and Quorum Quenching in Pathogenic Staphylococcus aureus and Pseudomonas aeruginosa. Int. J. Of Advaced Biotechnology and Research. 3(1):515-522.
[43] Hassan J, Crawford R J, Ivanova E P. Antibacterial Surfaces the Quest for a New Generation of Biomaterials. Trends in Biotechnology. 2013; 31(5): 295-304.
[44] Hossain F, Perez O S J P, Hwang S, Roman F. Antimicrobial Nanomaterials as water disinfectant: Application, Limitations and Future Perfective. Science of The Total Environment. 2014; 466-467: 1047-1059.
[45] Zhang Y, Yan M, Gao P, Jiang J, Zhang G, Li J, Shuang S. Immobilization of uricase-gold nanoparticles composite nanomaterial on a biofilm and its application to determination of uric acid. Applied biochemistry and microbiology. 2015; 51(4), 470-478.
[46] Manjumeena R, Duraibabu D, Sudha J, Kalaichelvan P T. Biogenic nanosilver incorporated reverse osmosis membrane for antibacterial and antifungal activities against selected pathogenic strains: An enhanced eco-friendly water disinfection approach. Journal of Environmental Science and Health. Part A. 2014; 49(10): 1125-1133.5