ENVIRONMENTAL FACTORS INFLUENCING BACTERIAL BIOFILM FORMATION AND INACTIVATION

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ABSTRACT

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Objective of this study was to evaluate effect of UV-C radiation (0, 1.16 and 3.21 kJ/cm^2), pomegranate (*Punica granatum*) seed essential oil (PGEO) and lemon (*Citrus lemonum*) plant essential oil (CLEO) on decontamination of *Candida albicans, Listeria monocytogenes, Staphylococcus aureus* and *Esherichia coli* O157:H7 biofilms formed at +4°C and 20°C on polystyrene, stainless steel and glass surfaces.

After 16, 32 and 64 sec UV-C treatment 0.24 log CFU/cm^2, 1.61 log CFU/cm^2, 1.59 log CFU/cm^2 reductions were achieved in the numbers of *C. albicans* biofilms formed at 20°C on polystyrene. In the numbers of *S. aureus* biofilms formed at 20°C on polystyrene 0.99 log CFU/cm^2, 1.9 log CFU/cm^2, 3.91 log CFU/cm^2 reductions were obtained after 16, 32 and 64 sec UV-C treatments, respectively. In general *C. albicans* biofilm formed at 20°C on stainless steel was found as the most UV-C resistant biofilm. CLEO inhibited the growth of *C. albicans, L. monocytogenes* and *S. aureus* at MIC values of 186 µg/ml, 103.5 µg/ml and 103.5 µg/ml, respectively.

The results of the study showed that UV-C radiation and CLEO can be used as an anti-biofilm agent to control or to prevent biofilm formation of foodborne bacterial pathogens. It was suggested that UV-C radiation and CLEO treatments have potential as a biofilm control interventions for the food industry.

ÖZET

BAKTERİYEL BİOFİLM OLUŞUMU VE İNAKTİVASYONUNU ETKİLEYEN ÇEVRESEL FAKTÖRLER

Bu çalışmanın amacı UV-C radyasyonunun (0, 1.16 ve 3.21 kJ / cm^2), nar (*Punica granatum*) çekirdeği esansiyel yağı (PGEO) ve limon (*Citrus lemonum*) bitkisi esansiyel yağının (CLEO) +4°C ve +20°C'de polistiren, paslanmaz çelik ve cam yüzeylerde oluşturulan *Candida albicans*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Esherichia coli* O157:H7 biyofilmlerinin dekontaminasyonu üzerindeki etkisini değerlendirmekti.

16, 32 ve 64 saniye UV-C uygulanmasından sonra, polistiren üzerinde 20°C'de oluşturulan *C. albicans* biyofilmlerinin sayısında sırasıyla 0.24 log CFU/cm^2, 1.61 log CFU/cm^2, 1.59 log CFU/cm^2 azalma elde edildi. Polistiren üzerinde 20°C'de oluşturulan *S. aureus* biyofilmlerinin sayısında 16, 32 ve 64 saniye UV-C uygulanmasından sonra sırasıyla 0.99 log CFU/cm^2, 1.9 log CFU/cm^2, 3.91 log CFU/cm^2 azalma elde edildi. Genel olarak, paslanmaz çelik üzerinde 20°C'de oluşan *C. albicans* biyofilminin UV-C'ye en dayanıklı biyofilm olduğu bulunmuştur. CLEO, *C. albicans*, *L. monocytogenes* ve *S. aureus*'un büyümesini sırasıyla 186 µg/ml, 103.5 µg/ml ve 103.5 µg/ml MIC değerlerinde inhibe etti.

Çalışmanın sonuçları UV-C radyasyonunun ve CLEO'nun gıda kaynaklı bakteriyel patojenlerin biyofilm oluşumunu kontrol etmek veya önlemek için bir anti-biyofilm ajanı olarak kullanılabileceğini göstermiştir. UV-C radyasyonu ve CLEO uygulamalarının, gıda endüstrisi için biyofilm kontrol müdahaleleri olarak potansiyeli olduğu öne sürülmüştür.

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

Figure **Page**

LIST OF TABLES

Table **Page**

LIST OF ABBREVIATIONS

subsp. :	Subspecies
µm :	Micrometer
min :	Minute
g :	Gram
mg :	Miligram
L :	Liter
µl :	Microliter
h :	Hour
rpm :	Round per minute
EPS :	Exopolysaccharide
cfu :	Colony forming unit
EDTA :	Ethylene Diamide Tetra Acetic Acid
TE :	Tris-EDTA
TAE :	Tris Acetate EDTA
SDS :	Sodium Dodecyl Sulfate

CHAPTER 1

INTRODUCTION

Solid surfaces containing nutrients, ions and other organic substances, such as in nature or food systems, microorganisms adhere to the surface by ensuring their viability and growth. If this situation is not interfered, microorganisms will begin the formation of biofilms, thereby causing blockages in the pipes in the system. Two terms expressing growth and biological contact on contact surfaces are known as biofilm and biofouling. Biofilm generally consists of extracellular polymeric materials (EPS) made of polysaccharides and the microorganisms that make them. The purpose of microorganisms to form biofilms is to act as a trap for nutrients and to protect them from other enemy factors or environments. Biofilm formation causes major problems and material losses in many industries. In particular, the systems they cause fear are the food industry and in process and waste-water distribution systems. Therefore, disinfection and cleaning is the priority of these systems in preventing or eliminating microbial accumulation.

As mentioned above, biofilm formation could be shown as causing problems in industries. Sessile organisms predominate in industrial and environmental relations. Generally, it might be the biofilm that forms inside the pipe, causing the decreased flow in the pipes. As a result of fouling, the product can become contaminated, heat transfer can decrease, and pipes can corrode due to the acid produced by the biofilm. Production is made in the food industry using different types of equipment, and it causes microbial contamination of these equipment in biofilms. Care should be taken to separate the biofilms from the surfaces they hold on, to prevent individual microorganisms from spreading to the area. If processing is carried out on biofilms with an inappropriate cleaning, it causes serious contamination of raw meat, as in the meat industry. This also indicates that decontamination procedures are inaccurate or incomplete. If contaminated raw meats produced in this way are not cooked properly or contaminate other kitchen utensils, they cause foodborne diseases and carry serious health risks. The problems are not only related to raw meat, but also pose a risk in the whole food industry and in all food processing materials, bands or all tools and equipment that come into contact with food. Even though preventing them gains importance, it is important to be able to

intervene after they occur. Therefore, quite in-depth knowledge and research is needed on biofilm.

1.1. Biofilm and Its Formation in Food Industry

The first microbial biofilms were discovered by Antonie van Leeuwenhoek using primitive microscopes on the tooth surface. A biofilm is defined as a microbially derived sessile community and are characterized by cells that are irreversibly linked to a substrate, interface or to each other. Cells have been reported to bind faster to hydrophobic surfaces (non-polar surfaces such as plastic) rather than hydrophilic surfaces such as glass or metal (Donlan, 2002). Stainless steel, glass and plastic are often used in the construction of food processing equipment and in food packaging. Regular mechanical or chemical cleaning can damage these surfaces. Microorganisms and organic substances can be collected in these areas and protected from disinfectants.

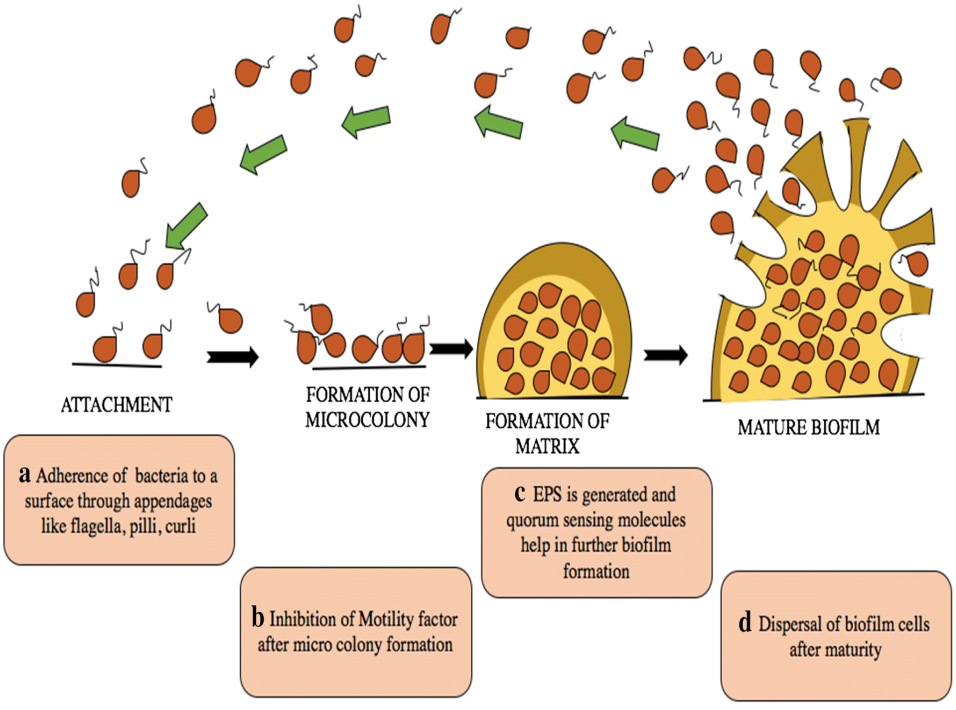


Figure 1.1. Biofilm formation stages; Step by step a, b, c and d.

Biofilms are known as bacterial populations that adhere to each other or to the surface within the matrix. These sessile organisms provide protection with an organic matrix. Biofilms could be created by all kinds of microorganisms, including pathogenic

microorganisms, when environmental conditions are appropriate. The tendency of bacteria to form biofilms is different from each other. For example; *Enterobacter*, *Staphylococcus*, *Pseudomonas*, *Flavobacterium* and *Alcaligenes* have higher tendency to form biofilm than others. Biofilms can be created on any surface, provided that the nutrients are abundant in the ecosystem. Depending on the growth stages, microorganisms can be found in two phases namely planktonic and sessile. While microorganisms live as free-floating individual organisms in the planktonic phase, they form a biofilm in the sessile phase and act as a closely integrated community by attaching to a surface (Figure 1.1). They prefer to live as sessile organisms because they have an exopolysaccharide (EPS) matrix where they will be protected from antimicrobial agents.

At first, biofilms were perceived as stable compact structures and evaluated in many cases as such. With the studies carried out today, it is understood that the biofilm is structurally porous, containing capillary water channels. The structure and architecture of the biofilm is closely related to the flows in the environment and the number and species of the different species they contain. In figure 1.2, where the complex architecture appears distinctly, the biofilm created belongs to a single culture. Water canals that could be seen between and below the micro colonies.

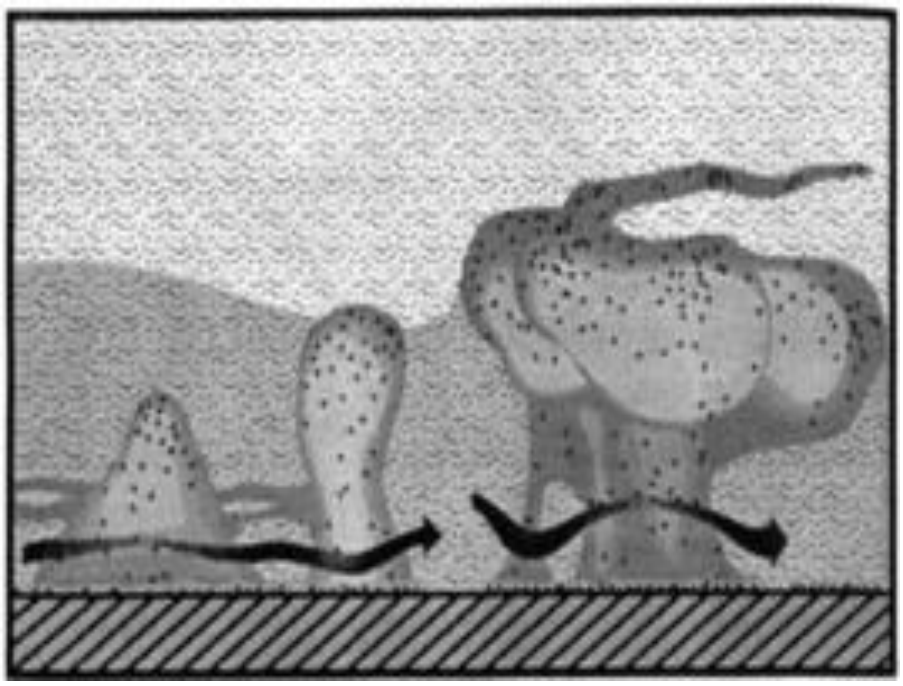


Figure 1.2. The architecture of a biofilm formed by a single-species biofilm based on data collected by CSLM of living biofilms. *Annual Review of Microbiology*, 49 (1995).

In biofilms, nutrients are delivered to microorganisms on the ground through special capillary tubes. In addition, the waste products they produce are secreted from the surface of the biofilm through these capillary tubes. Facilitation of transport is provided in two ways: water and passive diffusion. This facilitated transport also benefits the introduction of molecules for biofilm. The idea that oxygen is transported to the inner parts of the biofilm through water channels is a common belief. unfortunately, due to diffusion rules, it causes low oxygen in the inner parts of the biofilm. This explains how biofilm provides to keep various microorganisms (aerobic and anaerobic) together.

It changes cellular mass, water channels and extracellular polymers by constantly affecting the living cell distribution in the biofilm. While the structure and architecture of the biofilm for single cultures are species-specific, in multiple cultures, this occurs as substrate-specific. Due to the fact that there are microorganisms that show different adhesion and growth in heterogeneous biofilms, they often show irregularity due to differences in the architectural structure they create (see Fig. 1.2 as an example). Nevertheless, this architecture is the result of cooperation between microorganisms. Flow rate is a factor that determines the thickness of the biofilm. Reaching the maximum thickness of the biofilm is possible when turbulent and laminar flow is provided. The thickness of the laminar region is determined depending on the erosion in the turbulent zone and the availability of the substrate.

In biofilms, structures called as glycocalyx formed by Gram-positive and Gram-negative bacteria are integral parts. Glycocalyx is known as a capsule or slime, providing responsible forces that form adhesion and adhesion to surfaces that are of great importance for biofilm. The desired electrostatic interactions are performed with weak interaction such as van der Waals forces and hydrogen bonds. The immobilizing of the cells and the settlement of the slime cementing that the biofilm is in the stage when it matures. In the biofilm structure, the glycocalyx thickness ranges from 0.2 to 1.0 μm. The environmental conditions and growth environment of the biofilm control the flexible glycocalyx composition. The researchers have shown that for bacterial biofilms, the flexibility of the envelope provided is crucial in the response to changes in the growth environment. By using these mechanisms, pathogenic bacteria increase their chances of survival in extremely hostile and difficult conditions in the host they enter. Glycocalyx can cause changes in antibacterial molecules reaching their targets within the cells. According to some researchers, the glycocalyx matrix creates a highly effective resistance to large molecules such as antimicrobial components and proteins. In addition, this

physiological barrier acts against smaller peptides-defensins and their analogs. The slime changes free energy and charge on bacterial surfaces to reduce the transport of biocides. The effect of glycocalyx on diffusion barriers may vary depending on its solubility. It is known that glycocalyx accumulates up to approximately 20-25 percent of the weight of the antibacterial substance. In order to understand how antimicrobial agents are caught, extracellular alginate produced by *Pseudomonas aeruginosa* was investigated and its ability was found to be related to the anionic nature of the exopolymer. The fact that cationic substances are retainable in the matrix prevents these substances from penetrating on the bacteria in the biofilm. It has also been found that the mechanism of action of alginate is to inhibit their activity by binding positively charged biocides. It was found that excessive alginate produced by *P. aeruginosa* biofilms affected the resistance to antibiotic tobramycin treatment. On the other hand, it was observed that they penetrate the biofilms formed by *Staphylococcus epidermidis* in the environment where cefotiam, vancomycin and rifampicin antibiotics are exposed. This information tells us that its resistance to antibacterial compounds cannot be defined by the diffusion limitation created by the glycocalyx matrix. The presence of adsorption sites in the matrix is necessary for the glycocalyx to limit the passage of biocides. All binding sites in the glycocalyx matrix, after prolonged exposure to antibiotics, were saturated with medication, resulting in the killing of the *S. epidermidis* and *S. aureus* biofilm.

In addition to these, adsorption points in the glycocalyx can also be used for fixation of exoenzymes in the external environment. Thus, it can prevent the effect and penetration of sensitive drugs through immobilized enzymes. In addition, neutralizing enzymes synthesized by a member of communities with mixed species can provide protection in the biological layers, for all tested sessile populations. Exozymes continue to exist in the biofilm matrix, not only protecting the sessile population from certain agents, but also by clearing and breaking down the elimination metabolites of biocides, such as a substrate source.

1.2. Environmental Factors Influencing Biofilm Formation

Regardless of the environment in which the free cells are, they can form a biofilm here either in a solution or in nature or in the food industry when they find a surface. Biofilm formation is an event that can be called a complex that is affected by many factors.

The architectural properties and functionality of the biofilm are affected by the features, surface, environmental conditions and microbiological condition of the environment in which it is located. (Figure 1.3).

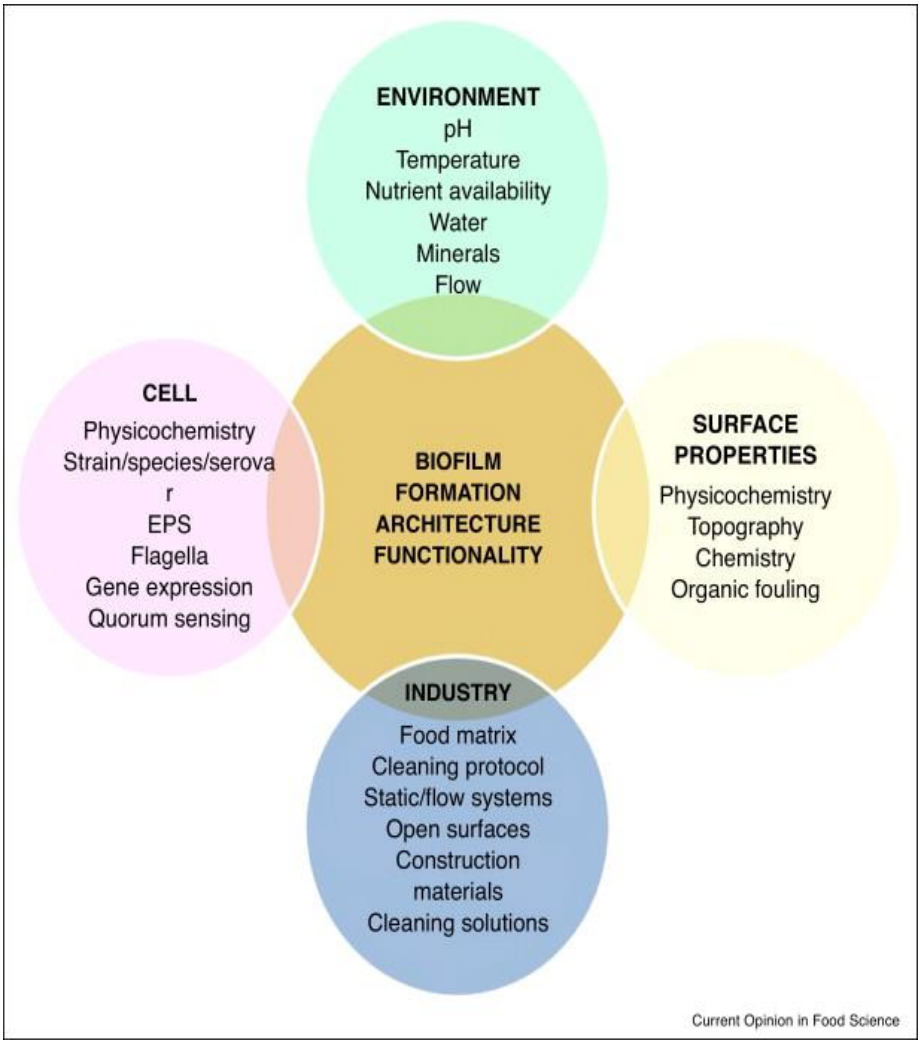


Figure 1.3. Factors affecting biofilm formation.

It is known that what influences the behavior of planktonic cells (resistance, toxin production, growth, etc.) is environmental conditions under which bacteria develop and grow. Water activity, interaction with the host, redox potential, nutrients, temperature, osmolytes, antimicrobials, pH, etc. factors such as these are examples of environmental conditions. At the same time, the physiological state of the cells in the biofilm may change, influenced by environmental conditions. Since biofilm formation requires contact between the substrate and bacterial cells, it can affect both the substrate properties and the bacterial properties. In addition, Table 1 shows the effect of environmental conditions in food processing on biofilm formation.

Table 1.1. Environmental factors in food processing and their effects on biofilm formation.

Factor		General effect	References
Chemical composition of substratum	Texture	Rough surface of substratum favors biofilm initiation	(Mariani et al., 2011; Wirtanen et al., 1996)
	Hydrophobicity	Hydrophobic material surfaces favor attachment of bacteria with hydrophobic properties	(An and Friedman, 1998; Katsikogianni and Missirlis, 2004)
	Surface charge	Opposite surface charges of substratum and cell favor attachment	(Morisaki and Tabuchi, 2009)
Temperature		a) Lower temperatures lead to more uniform properties of polysaccharides, which stimulate biofilm formation b) Lower temperatures decrease cell surface hydrophobicity level, leading to a lower biofilm formation	(Chavant et al., 2002; Di Ciccio et al., 2015; Garrett et al., 2008)
Oxygen concentration		Decrease of oxygen within biofilms reduces bacterial metabolic activity and inhibits bacterial growth	(Anderson and O'Toole, 2008)
Hydrodynamic effects		Higher shear rates decrease bacterial attachment bacteria, but increase density and thinness of biofilms	(Katsikogianni and Missirlis, 2004)
Food matrix composition		a) High osmolarity of food matrix inhibits biofilm formation b) Influence of pH and ionic strength on biofilm formation through changes in surface hydrophobicity and charge	(Jubelin et al., 2005; Katsikogianni and Missirlis, 2004)
Microbial interactions		Variable effect	(Bridier et al., 2015; Van Houdt and Michiels, 2010)

1.2.1. Contact Surfaces

Materials applied for food contact surfaces include polycarbonate, glass, polypropylene, ceramic, stainless steel, polycarbonate, polystyrene, teflon, nitrile rubber, wood, aluminum and titanium. In general, biofilms can evolve on the surface of any of these materials. Many factors play a role in adhesion to layers. Physicochemical properties such as the texture of the floor, the surface charge formed, and its hydrophobic property can be given as examples (Donlan and Costerton, 2002). In contrast, these physicochemical factors can be affected and altered by environmental conditions. It causes differentiation in food matrix such as temperature, pH, nutrient composition.

Among these surfaces, the most commonly used material in food processing equipment is stainless steel due to its mechanical strength and resistance to cleaning agents and corrosion. In addition to all this, there are some wrong applications to facilitate the binding of bacteria. For example, mechanical cleaning allows bacteria or organic residues to easily bond to the surface by causing scratches, minor deformations and even prongs. (Wirtanen et al., 1996). In particular, the porosity and absorbency of the wood material allows the retention of organic material and bacteria and increases the formation of biofilms on its surface.

In addition, slicing materials, one of the many tools used in food processing determined to cause contamination, constitute an important factor in biofilm (Srey et al., 2013). In particular, studies on frozen ready foods have revealed that smoked fish and slice meat products are the most common, contaminated foods with pathogenic microorganisms. More importance should be attached to the development of new model food surfaces with today's technologies for food preservation.

1.2.2. Temperature

Temperature is a highly variable parameter in the food industry, for example; Even temperatures above 4 ° C to 15 ° C in processing plant environments, -18 ° C in freezers, 4 ° C in refrigerators, 72 ° C in milk pasteurizers, and over 100 ° C in durable materials such as pipes can be applied.

Temperature can cause many changes in the cell; the physiological state of the cell, the physical properties of the compounds in and around the cells, and properties of the substratum. Incubation temperature also changes cell physiology. Bacterial growth rates are at a different optimum temperature for each strain. The bacterial rate decreases at temperatures lower than optimum temperatures. high temperatures may compromise microbial viability.

Increases in nutrient intake cause rapid biofilm formation because nutrient metabolism depends on the presence and activity of enzymes. The properties of the cell surface, such as hydrophobicity, charge, and electron acceptor-donor, may also be affected by the incubation temperature (Briandet et al., 1999). The main effect of temperature affects bacterial growth rather than planktonic growth rate, increasing

biofilm formation. Therefore, it is thought that the effect of temperature change on bacterial cells has a large effect on biofilm formation relative to other affected structures.

1.2.3. Microbial Interactions

In biotic factors, such as interactions between different microbial populations, it could affect biofilm formation along with abiotic environmental factors. One of the factors that greatly influences the development of bacterial biofilms and their initial formation on the surface is the coexistence of two or more types of bacteria. As explained in the previous sections, a wide variety of bacteria are found in food processing environments and it is observed that they form biofilms on the substrate. Therefore, complex partnerships between different bacterial species create more complex structures with a dynamic network for biofilm. In particular, biofilms containing two or more species show greater resistance to antimicrobials than biofilms containing one species. The resistance formed here is not provided with especially selected extra resistant species, but with the protection offered by a resistant bacteria contained in the biofilm to all other bacteria groups in the biofilm. In addition to this protection provided; it might cause an increase in the production of more biomass and a longer pathogen effect for a long time.

However, this relationship is not always positive for all partners in the community. it is often the case that biofilm formation is inhibited by one of the bacteria that make up the community. For example; the formation of biofilm created by *L. monocytogenes* has been shown to decrease in the presence of bacteriocin-producing Lactococcus lactis (Van Houdt and Michiels, 2010).

1.2.4. Oxygen Concentration

Microorganisms live in aqueous environments depending on the exchange of molecules by diffusion; they can live freely in the presence of water, and when they form biofilms, they contain some water, attach to inanimate objects and are in contact with host tissue. Consequently they experience the amount of oxygen in their immediate surroundings. Moreover, biofilms contain nutrients and a concentration of metabolic products, among which is the best known and studied oxygen. Oxygen acts as the last electron acceptor in aerobic respiration (Willey et al., 2011). In the absence of oxygen, a microorganism usually uses another organic molecule as the final electron acceptor.

However, more than the energy obtained by fermentation is obtained through aerobic respiration. Therefore, oxygen availability becomes important as bacterial energy production will affect biofilm formation. Creating micro-environments in biofilms leads to late growth of bacteria, such as oxygen zones restricted by biofilms and deprived of nutrients. The reduction of some important factors in biofilm limits the metabolic activities and growth of bacteria to a great extent. Oxygen deficiency and nutrient deficiency are the most important ones. This may result in cell detachment because it may not supply the energy necessary to maintain cell attachment.

In addition, oxygen, which affects surface hydrophobicity, also changes hydrophobic interactions on the surface. In general, a decrease in hydrophobicity indicates an opposite increase in surface oxygen.

1.2.5. Hydrodynamic Effects: Static and Flow Conditions

In the food industry, there may be both flow conditions such as pipes or corrugated pipe and static conditions such as a processing table or knife. Fluid shear can affect the physical properties of biofilms, such as binding strength and cell density: isotropic structures can occur under static or low flow conditions but the direction of the filamentous cells or where the cell groups are formed determines the higher flow.

It is generally believed that what makes biofilms denser and thinner is higher shear rates, higher separation forces that allow the number of bacteria to decrease. Despite the inability to explain the molecular response to fluid flow, the role of hydrodynamics in biofilm development and structure is of great importance.

However, it is the low shear force that causes cells to spread and colonize more of the substrate area than under high shear stress, which forms tight microcolonies. As a result, it is a flow that would allow for a stable interaction between bacteria and substrata, which would identify preferred colonization sites.

1.2.6. Food Matrix Composition

Food matrix components affected bacterial binding in food processing environments. By initially reducing the remnants of meat products, biofilm formation in stainless steel, wall and floor materials was reduced by *L. monocytogenes*, but biofilm

cell numbers and resistances increased in later stages (Somers and Wong, 2004). In addition, *L. monocytogenes* increased the formation of biofilms in nutrient-poor media rather than nutrient-rich medium.

In addition, in various pathogenic and laboratory strains of *E. coli* and clinical isolates of a number of *Enterobacteriaceae* and *Bacillus subtilis*, environmental glucose and catabolite pressure inhibits multi-layer biofilm formation (Karatan and Watnick, 2009). Biofilm formation of *B. subtilis* is activated when glucose is at low concentrations, but is inhibited at high concentrations (Stanley et al., 2003)

In many cases, although there is a high osmolarity of the food matrix among those that inhibit biofilm formation, this effect may be due to the type of osmolyte. For example it was shown that 100 mM NaCl in growth medium repressed transcription of curli genes by the transcription factor (CpxR) (Jubelin et al., 2005). On the contrary, it shows us that the environmental signal is ionic strength rather than osmolarity, that adding sucrose in similar concentrations does not produce the same effect.

Ionic power that controls how electrostatic interactions could be attractive or repulsive. Both bacterial cells and substratum surface are negatively charged at neutral pH. Therefore, two opposite types of interaction are observed under these conditions: van der Waals attractive interaction and electrostatic repulsive interaction. Depending on which of these interactions is predominant, it is determined whether bacterial cells will adhere to the surface. Increasing the ionic strength in the food matrix can reduce electrostatic interactions by the added salt due to the separation effect of the surface charge produced (Moreno-Castilla, 2004). Morisaki and Tabuchi (2009) found that the rate of attachment to the glass surface increases with increasing ionic strength of all bacterial cells tested and becomes constant at a certain level. Increased binding might result from the reduction of the energy barrier between bacterial cells and glass surface from ionic strength.

Bacterial gene expression, which affects the physicochemical properties and bacterial adhesion of cell envelopes, could change growth under acid or alkaline conditions. Chagnot et al. (2013) showed that prominent specific bacterial adhesion of *E. coli* O157:H7 to muscle proteins at pH 5.5 was not observed, and maximum adhesion occurred at pH 7. Similarly, some studies showed that *L. monocytogenes* strains had a lower adhesion ability at pH 7, not at pH 5. Less hydrophobic cell surface and down regulation of flagellin synthesis have been associated with reduced adhesion of *L. monocytogenes* to surfaces. In addition, changes in pH, which modulate electrostatic

interactions between the substratum and bacteria through changes in surface charges, lead to the separation or protonization of electrolytes.

Therefore, the properties of the food matrix, which mostly change the surface properties of bacteria and materials, affect bacterial adhesion. In addition, changes in bacterial physiology associated with surface attachment can be caused by the composition and concentration of the food matrix.

1.3. *Listeria. monocytogenes* and Importance

L. monocytogenes are facultative anaerobic bacteria that are gram positive. In addition to being psychotropic, they are pathogenic in human beings. Although rare, they can have serious consequences when they make foodborne infections.They show a fairly common distribution in nature. They are difficult to control in the food industry because they show their tribe at very wide temperatures. These temperatures include cooling temperatures (Fenlon, 1999).

1.3.1. Environmental conditions & *L. monocytogenes* biofilm formation

L. monocytogenes is one of the important species to be considered in the food industry in terms of colonization and its continuity in its environment for years (Chasseignaux et al., 2001; Latorre et al., 2009; Lundén et al., 2003; Vogel et al., 2001). It has some important advantages that ensure its growth and continuity in its environment. Thanks to these advantages, it can maintain its vitality both in food processing and on food. At the beginning of these advantages, salinity, low temperature and drying tolerance can be given. It has been shown to be present at all contact points in food processing (Lundén et al., 2003; Tresse et al., 2007; Vogel et al., 2001). The complex and holistic structure of the machines used makes it difficult to remove *L. monocytogenes* from the environment. There may be harsh chemical methods or regeneration of the belts for cleaning the machines. Because some tapes may not be able to be removed, the use of harsh chemicals can become inevitable.

In previous studies, it has been shown that *L. monocytogenes* in stainless steel, which is one of the most preferred materials in the food industry, especially in food processing, performs biofilm formation for a long time (Figure 1.4).

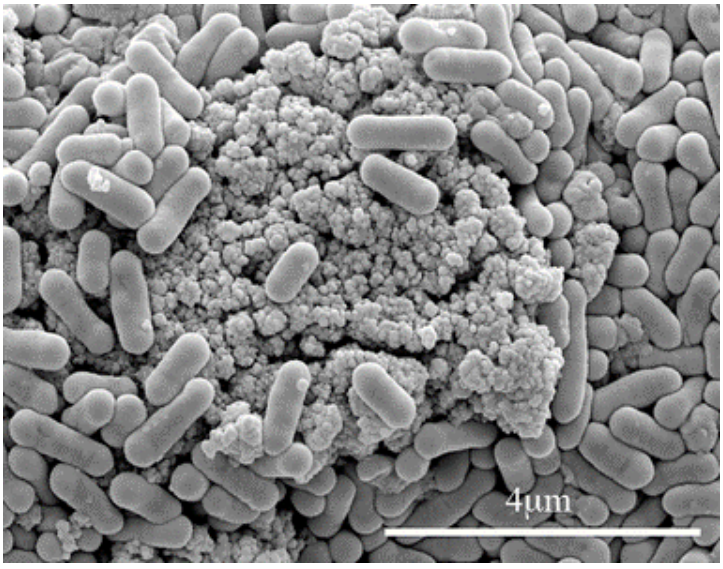


Figure 1.4. *L. monocytogenes* biofilm.

1.4. *Staphylococcus aureus* and Importance

Staphylococcus aureus is a pathogenic bacterium in the gram-positive group that threatens human health in many countries. (Chambers and De Leo, 2009; De Leo and Chambers, 2009). This bacterium causes acute or chronic diseases with a wide variety of features. The cause of this type of disease is due to the pore-forming toxins, superangens and virulence factors that provide tissue destruction. Like many, this pathogen has the ability to create biofilms in bacteria. The main benefit of biofilm is that it inactivates the immunity of the host and creates protection or resistance against antibiotics (Zimmerli et al., 2004; Costerton, 2005; Hall-Stoodley and Stoodley, 2005; del Pozo and Patel, 2007). In order for S. aureus to form biofilms, certain conditions must have occurred. Although it is known that biofilm formation is observed if many factors are met, we do not have a clear information about the mechanisms that ensure its complete formation and dispersion (Boles and Hors will, 2011; Kiedrowski and Horswill, 2011).

1.4.1. Environmental conditions and *S. aureus* biofilm formation

S. aureus is an organism that can adapt to various conditions and live like biofilms in different environments. Their production of biofilms is actually seen as an important virulence factor. The biofilm of *S. aureus* on the surfaces poses great risks in

terms of contaminating food, especially in the food industry. Researches made in world outbreaks, especially due to food, have proved to be found in food processing plants (Balaban & Rasooly, 2000; Braga et al., 2005; Hamadi et al., 2005; Marques et al., 2007; Nostro et al., 2004; Oulahal et al., 2008; Rode et al., 2007). Humans can be carriers of this bacterium. Can be seen in the nose, throat and skin. In this case, it may infect products in food processing workers (Gutierezz et al., 2012). The biofilms they form can have a multi-layer structure and provide protein expression (Archer et al., 2011). It is important for the food industry to know the various conditions that *S. aureus* has survived and the mechanisms of their reproduction.

The studies were generally done for *Staphylococcus* strains, which are problematic in medical devices and medicine, and the biofilm effects in this field are discussed. Regarding the biofilm it creates in the field of food, there is both a lack of literature and the amount of *Staphylococcus* isolated is not sufficient. It is clear that we need more studies and knowledge in order to control the biofilm that *S. aureus* creates, especially in the food industry.

The researchers have suggested that some factors remain uncertain, for example temperature, while some factors, such as hydrophobicity, provide biofilm resistance. The positive effect of hydrophobic surface on biofilm formation was compared with hydrophilic surfaces and the results revealed the difference of hydrophobic surfaces (Cerca, Pier, Vilanova, Oliveira, & Azeredo, 2005; Pagedar et al., 2010). it was found that single acting is not hydrophobicity, in fact it is one of the main factors in the formation of biofilm (Figure1.5) in surface roughness (Arnold & Bailey, 2000; Giaouris et al., 2014; de Jesus Pimentel-Filho et al., 2014; Katsikogianni et al., 2006; Lee et al., 2013; Tang et al., 2011).

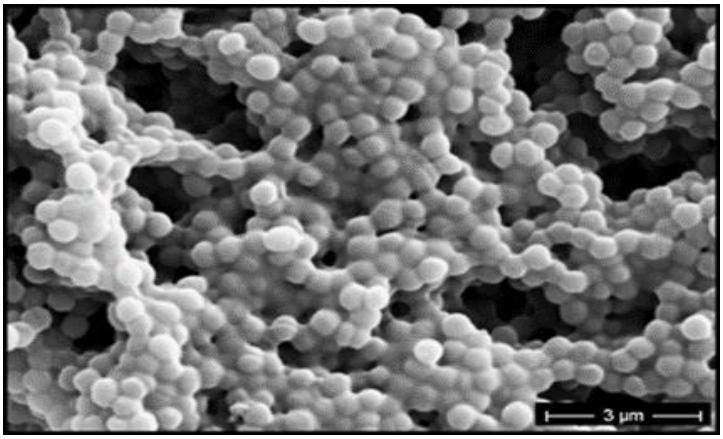


Figure 1.5. *S. aureus* biofilm.

1.5. *Candida albicans* and Its Importance

Candida is one of the best known fungal pathogens. They can use humans as hosts and they are known to be found in 50-60% of the oral cavities (Samaranayake, 1990). It has been reported that the rate of oral candida infections belonging to mixed cultures reaches not less than 10% -15% and they are generally related to C. albicans and C. tropicalis species (Jin et al., 2003). These fungi cause persistent oral systemic infections in patients, especially after chemotherapy methods used in the treatment of cancer and diseases affecting the immune system (HIV).

1.5.1. Environmental conditions and *C. albicans* biofilm formation

Candida species are low low virulence yeasts. Even so, it can form biofilms on tooth surfaces, acrylic prosthesis surfaces, together with other yeasts and bacteria, and develop mechanisms that increase pathogenic properties. (Thein et al., 2009; Weerasekera et al., 2017). *C. albicans* is known to form biofilms both in vitro and in vivo. When these oral biofilms they produce are not prevented from growing and the host immunity is low, they become macroscopically observed in the mouth called thrush (Weerasekera et al., 2016; Mukherjee et al., 2005; Bizerra et al., 2008). In addition to these, it is fermented glucose and sucrose in diets that increase tooth decay and various diseases and encourage *Candida* to form biofilms (Figure 1.6) and adhere in the mouth.

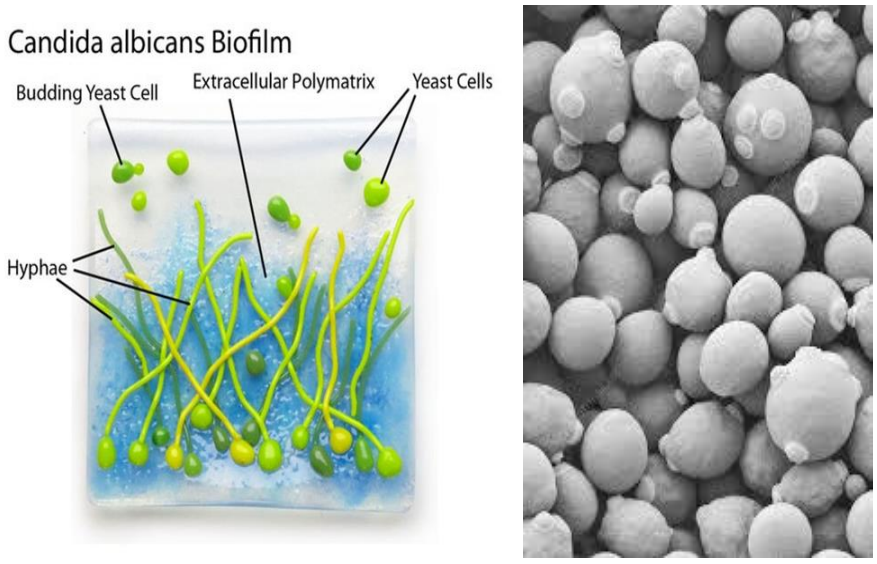


Figure 1.6. *C. albicans* biofilm.

1.6. Biofilm Resistance

In order to neutralize pathogenic microorganisms, disinfectants, which are chemical substances, are used in almost all kinds of microorganisms, especially on inanimate objects. Although the disadvantages of disinfectants can not show specific effects against more than one target, antibiotics are chemotherapeutic drugs that manage the metabolic process in specific structured microbial cells or interacts that are mostly used to control infections (Meyer and Cookson, 2010). Depending on the type of biocide used, their mode of action has been extensively described in the reviews (McDonnell and Russell 1999; Russell, 2003). Cytoplasmic membrane, DNA, RNA, cell wall or outer membrane, functional and structural proteins and other cytosolic components form an important structural target in Gram-negative or Gram-positive bacteria. Disinfection types are used for controlling biocontamination, especially for industrial, medical and domestic environments.

These applications for biocides can of course affect and eliminate the contamination to a great extent, but as mentioned earlier, they may pose a risk to public health as they cannot affect all microorganisms. Since this situation is of serious importance, various reports have shown that there are microorganisms that survive after disinfection of both medical (Deva et al., 1998; Martin et al., 2008) and domestic (Cooper et al., 2008) and industrial environments (Bagge-Ravn et al., 2003; Weese and Rousseau, 2006; Stocki et al., 2007). The resistance of microorganisms to various disinfectants increases in parallel with their biofilm formation. When they find moist media, most microorganisms are capable of producing an extracellular matrix (EPS), which is made up of nucleic acids, proteins and exopolysaccharides (Costerton et al., 1995; Branda et al., 2005; Hoiby et al., 2010).

It is well known that the cells that pass from the planktonic phase to the biofilm matrix form different phenotypes and develop resistance to biocide combat by developing specific features (Nett et al., 2008; Smith and Hunter, 2008; Wong et al., 2010). Since this situation differs according to planktonic or biofilm cells that take into account the definition of resistance, it has become important to distinguish. The fact that it can be said to be resistant to a biocide is understood by the fact that the strain of a microorganism cannot be inactivated at doses and application times that enable other strains to be inactivated (Langsrud et al., 2003). It can be said that biofilm cells have a higher capacity

to resist compared to planktonic cells. It should not be neglected that this resistance that bacteria has created against biocides is actually obtained phenotypically or genetically (Langsrud et al., 2003; Russell, 2003). it is supported by the fact that this resistance, which we think is occurring in the biofilm, may be a tolerance, and that when the cells in the biofilm show physiological changes in adaptation to the environment, they may disappear or decrease significantly when they return to their previous state, namely, their planktonic forms (Russell, 1999). In addition, some variants seen are known to be highly stable in biofilms.

Expressing biofilm sensitivity may contribute to the distinction of resistance in biofilm from resistance in planktonic phase. In order to evaluate this sensitivity against disinfectants by comparing, some determined standards, such as planktonic cells, should be increased in biofilm cells. Some methods are set as standard, these are ASTM standard method (no. E2799-11), MBEC assay system (MBECTM assay system, Biofilm Technologies Ltd, Calgary, Alberta) and some standards used for planktonic cells can be adapted (Meylheuc et al., 2006; Ntsama-Essomba et al., 1997). To be consistent in measuring the resistance occurring in the biofilm, it can be evaluated by measuring the ratio of concentrations (Rc) or time (Rt) in the biofilm population showing the same decrease as the planktonic phase or by comparing the decreases after the factor causing the decrease.

The Rc or Rt values for particularly common biocides found in the sources shown in Table 1.1. Looking at the values for Rc and Rt coefficients, it is seen that they are between 1 and 1000 and 20 to 2160, respectively, so that they can be related to species and biocide so that high biofilm resistance is against different disinfectants. The difficulty in comparison is that due to the lack of standard protocols, the results of biocides on biofilms cannot be compared. The shortage of information globally and quantitatively is not sufficient to prevent surface contamination for biofilm resistance. Hence biofilm resistance created by biocides is a concern for researchers.

Biofilms are a three-dimensional collection of microorganisms fixed on the surface with a complex architecture containing proteins, polysaccharides and DNA. increasing antibiotic resistance and permanent type infection are often due to biofilm formation; in the host tissue and implants. With the type of infection caused by biofilm formation, it is very fast to spread to the whole system. Thanks to their exopolysaccharide (EPS) matrices, a biofilm-formed microorganism community is highly resistant to antibiotics applied. The drugs that are separated from the biofilm and used for those who

switch back to the planktonic phase are effective in preventing the recurrence of the infection, but the same is not true for microorganisms in the biofilm.

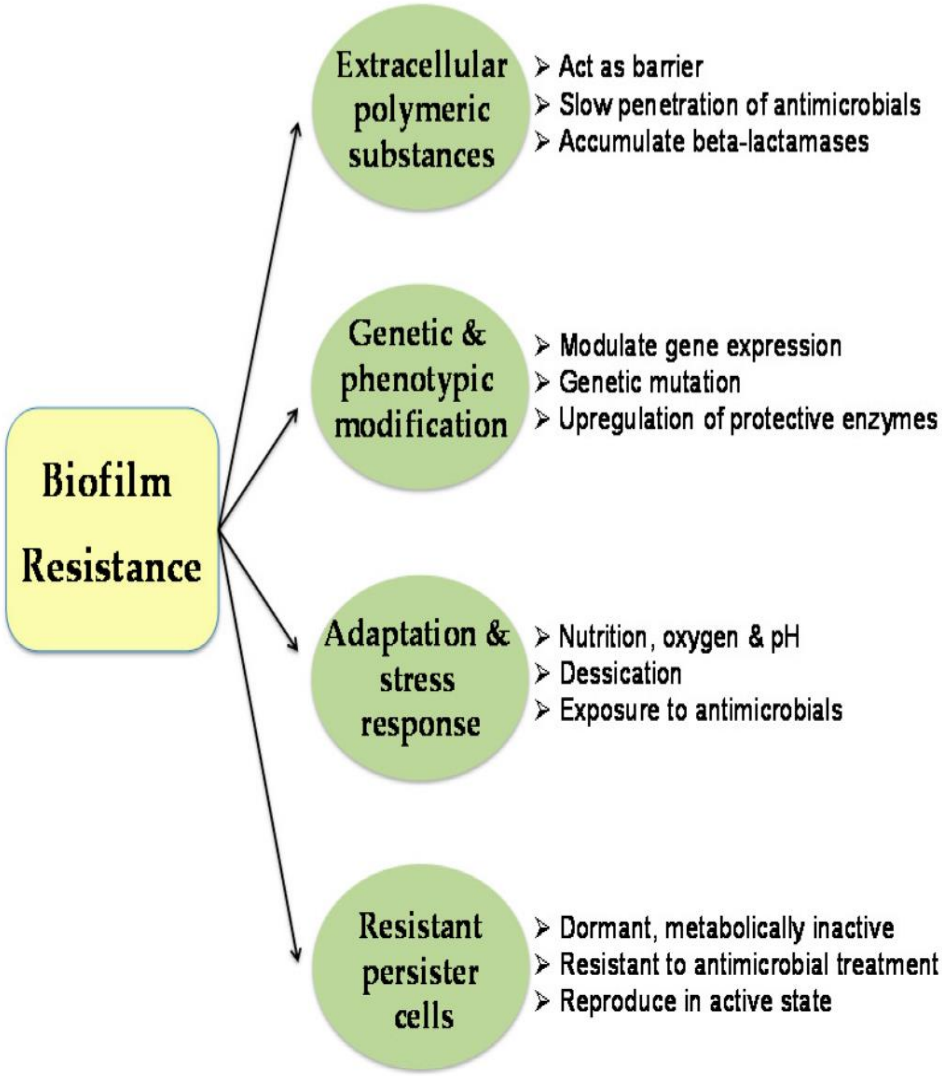


Figure 1.7. Biofilm resistance

Despite the high-dose drug administered, antimicrobial treatment, which is unsuccessful, is generally attributed to the resistance of the bacteria that make up the biofilm. Some sensitive bacteria, especially those without genetically antibiotic resistance, can develop approximately 1000-fold resistance when embedded in a biofilm. When the flow chart in Figure 1.8 is examined, it can be seen that the cause of resistance is related to more than one factor. EPS matrix has many features; phenotypic differences, intercell communication (quorum sensing) and efflux pump activation. Some of these factors are also classified as molecular mechanisms, biochemical factors and environmental factors.

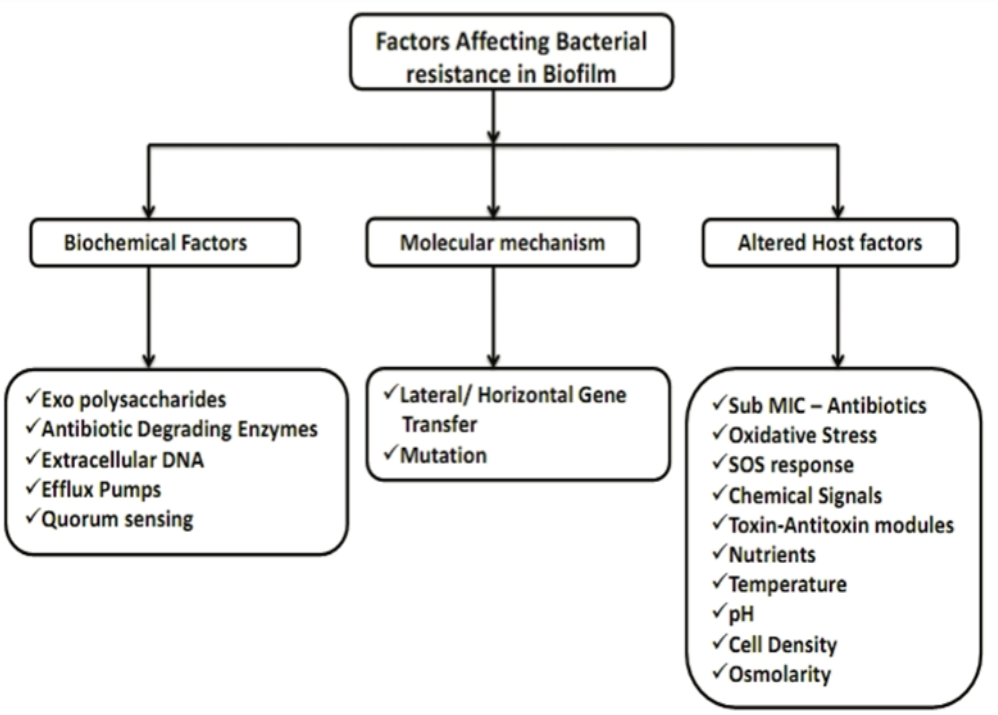


Figure 1.8. Factors influencing bacterial resistance in biofilm-associated bacteria.

1.6.1. Biochemical Factors Affecting Bacteria Resistance

Exopolysaccharides (EPSs). The EPS matrix formed by bacteria consists of extracellular DNA, intracellular adhesin, proteins and teichoic acid. The presence of different mechanisms in both the formation of the EPS structure and the formation of an encapsulated glycocalyx structure also affects the heterogeneous bacterial species that form the biofilm. The multi-layer EPS complex in *Staphylococcus epidermidis* is associated with the primary attachment of cells to the surface. There are three layers in the biofilm: the conditioning layer, the base layer and the outer surface layer. Apart from the many benefits it provides to bacteria, EPS also protects against antimicrobial agents, stress and many other environmental factors and supports the formation of a structural integrity.

It was suggested that the antibiotic resistance formed was caused by the limitations that occurred during the transportation of the drug, but it was understood that the antibiotics were positive and the EPS was negatively charged so that it reached a certain saturation, thus causing limited transportation. Besides EPS, it is in other protection systems, for example; peptidoglycan is known to act as a barrier to prevent the penetration of therapeutic agents.

Surface hydrophobicity and high unsaturated fatty acid (C16) are found more in membrane lipids of bacteria forming resistance in biofilm compared to sessile strains. Although its mechanism is not clear, changing surface properties are thought to increase bacterial resistance. One of the main components in EPS is Poly β (1.6) - N-acetylglucosamine (PNAG). This substance helps protect some microorganisms from the immune response created by the host, as well as provides resistance to antibiotics.

PIA is the main ingredient in biofilm that provides adhesion. The main ingredient that makes up PIA is glycan 1,6-(2-acetamido, 2-deoxy) D glucopyranosyl. PIA found in clinical bacterial strains also causes their virulence. It is understood that the pathogenesis seen in various medical devices is due to the multi-layer EPS (glycocalyx) complex formed by some microorganisms.

Enzyme-mediated antibiotic & heavy metal resistance. Some enzymes (β-lactamase) accumulating in EPS prevent the penetration of the drug with their antibiotic-disrupting properties. Also, some bacterial plasmids can induce heavy metal resistance with some enzymes. Metals corresponding to enzymatically reduced cations provide their induction. For example; Presence of arsenate and antimony provides resistance in wide variations in both metals where E.coli is present.

Extracellular DNA. The antimicrobial effect of the cell membrane is disrupted, and it is possible at physiological pH by chelating cations of DNA. It provides resistance to aminoglycosides and antimicrobial peptides as chelating agents by inducing various operons of some microorganisms with minimal DNA concentration outside the cell. In biofilms supplemented with extracellular DNA, the minimum biofilm eradication concentration (MBEC) is 8 to 64 times higher than the control biofilm of aminoglycosides and antimicrobial peptides (CAP).

Efflux Pumps One of the possible mechanisms is that it has a efflux pump with the resistance it creates thanks to the sealing. Basically the efflux pump consists of membrane proteins and extrudes antibiotics. The majority of efflux pumps studied in reducing the intracellular concentrations of toxic molecules include the main facilitating super family (MFS). The scarcity of oxygen and nutrients makes these pumps better expressed. Triple RND efflux pumps are encoded by a single operon. These pumps contain intermembrane, periplasmic and cytoplasmic proteins. It is these pumps that have taken a physiological role in the system, which is involved in the removal of amphipathic and other toxic molecules that denature the cell membrane. The arrangement of these pumps in the system plays a role in providing resistance for embedded bacteria. It has

been reported that the expression of genes encoding pumps does not relate to resistance in many studies. Induction of the pompar was demonstrated by the application of different antibiotics with a sub-minimum inhibitory concentration (sub MIC).

Quorum Sensing The high density of the cells in the high amount of the EPS matrix is also associated with the upregulation of the flow pumps, which is in the form of cell to cell signaling (quorum sensing). They are quorum sensing molecules that increase and increase bacterial resistance through horizontal gene transfer, but at the same time regulate biofilm functions in various ways, including pilus formation, and have a significant effect on cell density. While methyl peptides are QS molecules found in gram positive bacteria, N-Acyl Homoserine Lactones (AHL) are QS molecules seen in gram negative bacteria. Regulation of the QS system is provided by auto induction. The mechanism here interferes with the expression of the necessary genes of autoin-ducer, which binds to the response element by increasing intracellular AHL. Las and rhl are two separate quorum sensing systems. These create resistance to the immune response produced by the host and to chemotherapeutic agents. The first resistance is gained by the genetic structure of the bacteria. For example, mar operon reveals multiple antibiotic resistance in E.coli.

1.6.2. Molecular Mechanism

In biofilms, some properties with very high resistance can be caused by changing metal resistance genes and exposure to different drugs.

The c-diGMP-mediated response is among other signaling mechanisms that have occurred as a result of antibiotic administration. This can occur with different molecules in different microorganisms, for example; E. coli's ydeH (encoding diguanylate cyclase), P. aeruginosa's arr (encoding c-di-GMP phosphodiesterase). It should not be forgotten that the antibiotic should be at the MIC levels in the transport, besides the effective administration. An oscillation of sub-MIC values is sufficient for the initiation of biofilm formation. For example; The biofilm formed by P. aeruginosa is reduced by mupirocin. In contrast, P. aeruginosa, E. coli and S. epidermidis are known to have aminoglycosides, tetracyclines and lactam antibiotics that positively affect and increase biofilm formation.

The fact that the structure of biofilms is heterogeneous provides some features. An example of this structural feature is that gene transfer rate increases with the presence

of some strains. Some microorganisms (such as *S. aureus*) keep and increase the plasmid transfer rate in the horizontal direction. Increasing the mutation frequency increases the course of antibiotic resistance in parallel. Toxins and antitoxins respond to external stress by programming cell stress to maintain cell formation. K. pneumoniae increases the biofilm resistance by giving the rich antibiotic resistant plasmid to other microorganisms in the biofilm. A study showed that the biofilm developed resistance to antibiotics 48 hours after the resulting biofilm.

In the resistance-forming mechanism of the product formed as a result of gene mutation, there is prevention or reduction of antibiotic binding to proteins. The point to be kept separate is the antibiotic resistance that occurs naturally in the presence of a specific chemical. Not all mutations may produce this result. It may be random that the mutation creates antibiotic resistance.

1.6.3. Altered Host Factors

Subminimal inhibitory concentration of antibiotics. Antibiotics applied at very low concentrations (sub-MIC) play an important role in the formation of bacterial resistance. Biofilm not only prevents the penetration of antibiotics in the protection of microorganisms, but also provides protection at various antibiotic concentrations below the MIC value by creating different regions. The change of expression proteins maintains the resistance against the antibiotic. It is that they are exposed to sub-MIC levels that act as a signal molecule. It is these molecules that enable the activation of the SOS regulon. This happens during DNA damage. By controlling and activating some stress responses, they greatly alter the expression of many genes. The concentrations formed help differentiate resistance genes against antibiotics through transduction or transformation. The presence of the food source in the layers of the biofilm is different. While the lower layer is limited in terms of nutrients, the upper matrix is rich in food. In this case it leads to fasting mediated antibiotic resistance at the bottom.

Oxidative stress. The change in physiological conditions is actually due to oxidative stress, resulting in resistance. It enables the production of reactive oxygen species (ROS) to decrease by using antimicrobial agents of biofilm bacteria to suppress oxidative stresses. For example, the resistance of some species depends on the hydrogen sulfide (H2S) they secrete against the antibiotic. this also causes oxidative stress

responses (SOD). There are multiple factors that can cause bacteria in biofilms to form a highly tolerant phenotype. These are stress factors such as nutrients, heat or cold shock, pH, cell density and osmolarity, and cause induction of gene expression. There are some factors in bacteria that control and reduce the stress factor. It is able to reduce and manage sigma factors for oxidative stress.

1.7. Control Methods of Biofilm in Food Industry

Molds or moulds are fungi that reproduce in the form of multicellular filaments called hyphae (Moore et al. 2011).

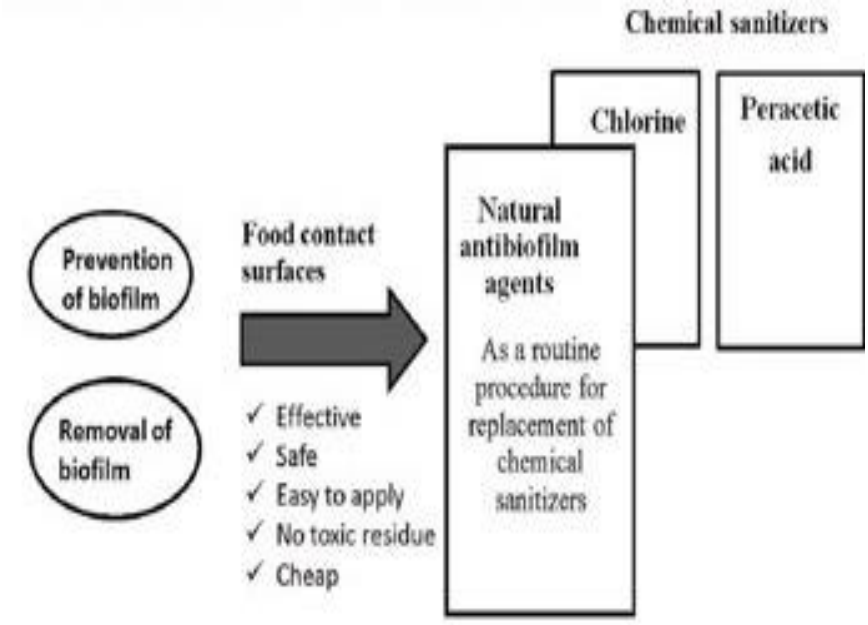


Figure 1.9. Control methods of biofilm in food industry.

1.8. Non-thermal Control Treatments of Biofilm in Food Industry

1.8.1. Shortwave Ultraviolet (UV-C) Light

In many areas, short wavelength UV-C light application has been well studied and has been in use for years. For example, it is used for decontamination of air, packages, surfaces and water sterilization, and even for wastewater disinfection. Investigations and improvements continued and paved the way for the application of these treatments and

methods in scientific and industrial fields. Non-ionic ultraviolet (UV) light, other than food, is 190-280 nm wavelength. It is also used in disinfection other than food surfaces and food contact surfaces. Operating range 245 nm UV-C light with no chemical residue. Affecting almost all microorganisms increases the importance of the studies. This method has subsequently attracted the attention of some important organizations and after the necessary examinations, the UV-C light has been approved by the U.S. Food and Drug Administration (FDA) as a means of purifying food products.

Usage areas of UV irradiation in industry do not end with counting. For example, materials with food contact surfaces such as containers, boxes, bottle caps, packaging are the main applications. (Bintsis et al., 2000; Cook et al., 2016; Kuse, 1982; Nicolas, 1995; Manzocco and Nicoli, 2015; Otto et al., 2011). For packaging and aseptic processes, it can be used not only with this method but also with some other methods that can create synergistic effects. In the destruction of bacterial spores, synergistic effect hydrogen peroxide (H2O2) and UV-C radiation are used together (Marquis and Baldeck, 2007). For polymer plastics, UV-C and ozone, which increase the effect, are used simultaneously (Ozen and Floros, 2001).

Decontamination for both non-food and food contact surfaces has been determined for some species and strains. nonpathogenic or non-O157 STEC E. coli strains such as ATCC 25922, 25253, 11775, 11229 and 8739 (Oteiza et al., 2005; Donahue et al., 2004; Guerrero-Beltrán and Barbosa-Canovas, 2005; Koutchma and Parisi , 2004; Milly et al., 2007; Murakami et al., 2006; Ngadi et al., 2003; Schenk et al., 2011).

E.coli strains, which show pathogenic feature in which the UV-C inactivation effect is evaluated, continue to be studied and investigated. (Murakami et al., 2006; Donahue et al., 2004; Gabriel, 2012; Gabriel & Colambo, 2016; Gabriel & Nakano, 2009; Gayán et al., 2011; Koutchma et al., 2004; Ngadi et al., 2003; Orlowska et al., 2015; Oteiza et al., 2005, 2010; Yin et al., 2015; Yoo et al., 2015; Yoon et al., 2018).

It shows us how the inactivation done lifts and clears the microbial load. The direct effect of DNA and RNA disrupted their transcription and replication, causing normal cell death.

Nucleic acids are known to absorb between 200 and 310 nm. Exposure of DNA and RNA to UV-C light at these wavelengths affects the bond between some bases in these molecules, causing the formation of pyrimidine dimers (Figure 1.10). Effect of UV-C light on DNA in the form of single-strand breaks.

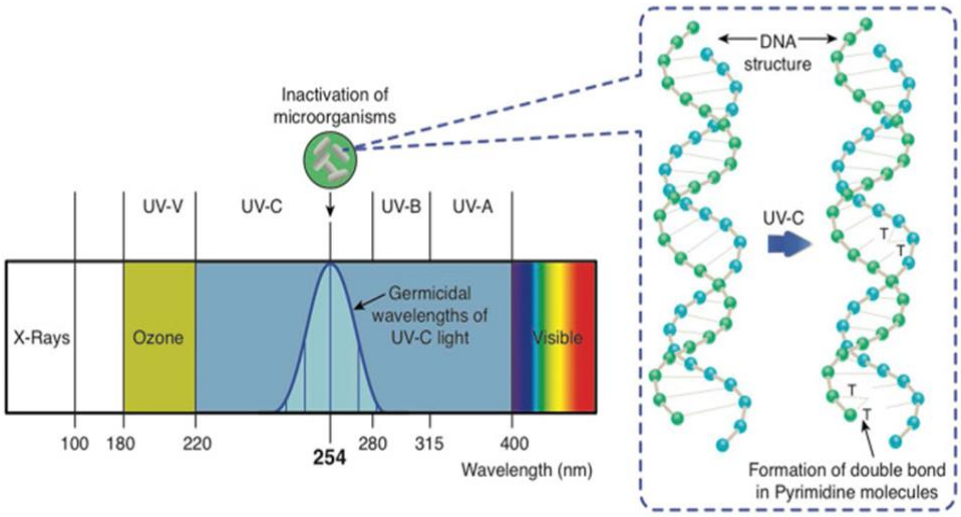


Figure 1.10. Effect of UV-C light on DNA in the form of single-strand breaks.
(From: Barba et al. (2017) in Trends in Food Science 66, 20-35)

Formation of dimers prevents proliferation. Microorganisms, which are prevented from growing, remain inactive. Photons produced by UV-C are absorbed by DNA bases, which causes cell death because inhibition of transcription and replication are created (Lopez-Malo and Palou, 2005). In UV-C liquid foods, some studies have been asked to cause a decrease in microbial load in liquid food. Although too many microorganisms have been studied, the main emphasis is *Yersinia pseudotuberculosis, Staphylococcus aureus, yeast Saccharomyces cerevisiae, L. innocua* and *Yersinia pseudotuberculosis* (Oguma et al., 2001; Turtoi and Borda, 2013). Interestingly, as determined by some researchers, *L. monocytogenes* showed higher UV-C resistance to juice (Gabriel and Nakano, 2009), solid surfaces (Rowan et al., 1999) and dairy products (Lu et al., 2011). There are some structural features on which this situation is based. The presence of a thicker peptidoglycan structure, separation from Gram negatives due to being Gram positive bacteria with higher chromosome density, and having a higher yield DNA error repair system make the difference for L.monocytogenes (Cheigh et al., 2012).

The chemical breakdown and cleavage of DNA is caused by the UV region and some antimicrobial effects. (Hallmich and Gehr, 2010; Mitchell et al., 1992; Oguma et al., 2002).

Some parameters are determinant factors that determine UV applications such as fluence, dose and uv fluence rate. Bolton and Linden (2003) defined the rate of fluency as the radiant power with an infinitely small area that flows from everywhere. Fluency is obtained when the exposure time and the fluency ratio are multiplied. The UV dose is not

synonymous with fluency. The dose actually refers to the energy absorbed in a way. From this point of view, it is revealed that there is a very small amount absorbed by microorganisms (Bolton and Linden, 2003).

Applied UV fluency is calculated based on the equation below. The UV fluency Applied Here is based on (t) a specified exposure time, (I_0) UV intensity to apply the product to the surface. Regardless of the material to be irradiated, the UV fluency in the application reflects the energy emission from the source. Thanks to the fluency information, the type of UV source can be determined and a correct power source is selected taking into account the efficiency.

$$H_{app} = I_0 \ (PF) \ t$$

The energy absorbed by the environment gives us the UV fluency absorbed and may cause photochemical reaction. It is possible to write the equation (1.1) in the way that the absorption coefficient α is zero:

$$abs = I_0 \ (PF) \ (RF) \ (DF) \ (1\text{-}10^{\alpha l}) \ t$$

The absorbed UV fluency is used to measure the deterioration of chemicals in the liquid medium.The target chemical or microorganism is expected to be affected when all of the absorbed energy is absorbed by the liquid when not all of the UV radiation is absorbed. In cases where microorganisms are not inactivated, we can understand that UV light is fully absorbed by the solution.

The energy absorbed and absorbed by the target component in the sample is the UV dose that is effective or delivered. It is calculated using the equation below;

$$D_{eff} = \int_0^t \frac{-dN / dt \cdot U_\lambda}{} dt$$

1.8.2. Essential Oil (EO) Treatment

Most of the natural products are used to contribute to human health. These products, such as essential oils, are used in food additives, medicines, cosmetics and various functional foods. Essential oils are an important alternative in a wide range of food industry. Instead of using chemicals or synthetics against food-borne pathogens or microorganisms that can degrade food, these natural products are safer to use for human health. Thanks to its antioxidant, antimicrobial and antimutagenic properties that can be considered beneficial on health, its usage areas are wide. Besides, they can be used as natural additives by inhibiting lipid oxidation (Burt 2004; FDA 2013; Zengin and Baysal, 2015).

Among those traditionally used in sanitation instead of natural essential oils; various chemicals, enzymes, preservatives, cooling or heat treatments can be shown. Since the use of chemicals can cause health problems, their use in food has been limited. The consumption orientation of people is towards natural products or less processed products. In this case, it is compulsory to find natural preservatives in food preservation by moving away from chemicals. In this case, it has increased the interest and studies in essential oils intensively.

The main reason they are used as preservatives in foods is that they are of natural origin and show antimicrobial properties. EOs are actually secondary metabolites. These are immune substances produced by the plant. Defense is provided against insects and various pathogens by producing EOs. EOs exhibit some properties that can vary depending on the plant from which they are obtained. They can be antibacterial, antiparasitic, antifungal. There are other uses, for example; They can act as an insecticide or antioxidant.

Antimicrobials have been used for more than 50 years. If the food antimicrobials are related to the resistances that they may have, studies are still not sufficient. Although the need for new antimicrobials is high, cost and application difficulties are obstacles, but natural compounds are more likely to overcome these challenges. Natural compounds can be tried in some areas where they are used without the need for legal approval. If we take America, for example, natural compounds are GRAS. In addition to these, they are

suitable for the new consumer demand. they are natural and do not contain inhibiting artificial ingredients.

Increasing research groups have shown that essential oils and their chemical ingredients are antimicrobial. for example; It has been shown on the food that clove oil inhibits the growth of *L. monocytogenes*. In addition, not only on a single food, that is, not only on meat, but also tried this oil on chicken meat and caught the same inhibitory effect on chicken sausages. It is one of the natural products that have been researched on vanilla. Apple food, which is another food, has been found to slow down reproduction, as shown in trials in TSB-YE broth, where it has a bactericidal effect on *L. monocytogenes*. It has also been proven in studies on food that vanillin is effective on *L. innocua* and prevents its spread. Considering all these results, it shows us that *L. monocytogenes*, which is found on various foods, is inhibited by natural antimicrobials.

The researches for these substances are in one-way progress and only data on their efficacy are produced. However, this cannot provide us with concrete information about the mechanisms of action of these compounds. In this case, it prevents the generation of information about potential resistances or even cross-resistances that may occur. The results so far show that the primary effect is on the cell envelope.

The researchers found that cinnamaldehyde and eugenol substances used to stop the increase in cellular ATP after glucose supplementation. It was also shown by the same researchers that a decrease was observed in the membrane ATPase activity in *L. monocytogenes* with the use of antimicrobial compounds of plant origin. It is an important finding for these natural antimicrobials to act in a small part of the targeted population without affecting the whole. It is also related to the fact that microorganisms that can survive non-lethal stresses may actually be associated with the non-lethal damage provided by the compounds and that they create a stress network by affecting other regulatory mechanisms (Wesche et al., 2009).

Although additional studies are needed, when the sensitivity of *L. monocytogenes* and *L. innocua* to various antibiotics in the presence of carvacrol and citral was examined, it was observed that they caused a positive increase in their antibiotic effects. It is necessary to understand how its susceptibility to clinical antimicrobials changes as their mechanisms of action continue to be investigated, especially to understand what conclusions can be made after natural antimicrobials administered *L. monocytogenes*. As research increases in this direction, it may cause a decrease in dependence on antibiotics and a decrease in the resistance created by bacteria against them.

1.9. Biofilm Formation by "Microtiter Plates"

Microbial biofilms are generally structures formed by microorganisms in a three-dimensional structure in a fixed area. They are mostly located in a matrix consisting of substances secreted by microorganisms that make up the community, and they are multi-cell systems consisting of prokaryotic or eukaryotic cells (Costerton et al., 1999). Although biofilm is not a complex structure as a structure, it goes through a multi-stage process as formation. Following microbial adhesion, a matrix of proteins, polysaccharides, extracellular DNA and various other molecules is formed. Since 1978, when the first biofilm definition was made, biofilm science and technology has been actively studied and attracted the attention of the public. It is well known that most known natural microorganisms do not live as free-floating organisms, but live in biofilms formed by adhering to surfaces. With the advancement of biofilm technology, biofilm architecture has become more comprehensible. Biochemical methods, new imaging technologies, development of ecosystem tools revealed the details of biofilm. A complete image of the biofilm structure can be perceived in three dimensions and detailed information of this structure can be reached at nano-scales (Neu & Lawrence, 2015).

Simultaneously, it is possible to obtain a deep knowledge and behavior about the genotypic and phenotypic diversity of the cells that make up the biofilm community, as well as their physiology. Since environmental conditions are of high importance in the formation of biofilms, it has led the biofilm device technology to develop in this direction to simulate these conditions in tests (Raes & Bork, 2008).

As the information about biofilm continues to increase, we can understand how they can be managed with the increase in the information of both its whole structure and its single cell structure. The deep knowledge provided is guiding in facilitating the control of harmful biofilms (such as clinical biofilms, food contaminants) and in developing and modifying biofilms (such as wastewater treatment, biological improvement) that can be beneficial. their realization cannot be met by a single disciplinary science.

Desired methods can be developed with a multidisciplinary approach. The selection of experimental platforms should be in the direction of obtaining the intended information. This requires that the chosen platform is chosen in a way that can serve this.

Making the experiment with the platform design provides its limitations and some advantages (Azeredo et al., 2017).

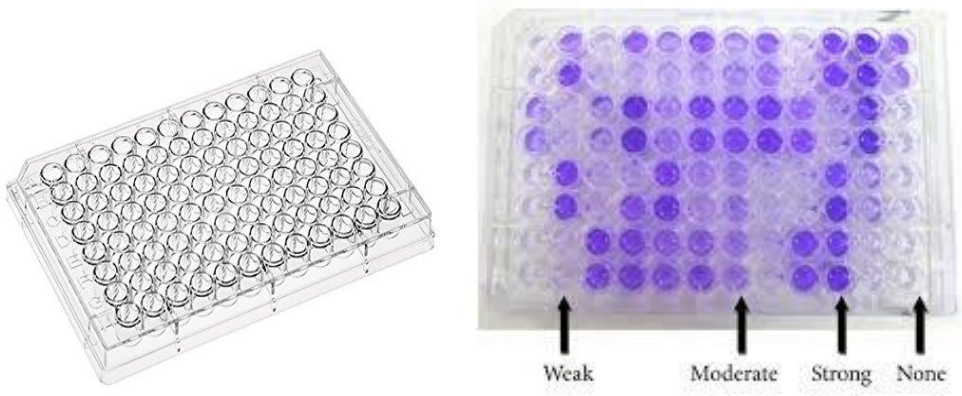


Figure 1.11. The microtiter plate (MTP) system.

In experimentally created biofilms, the first choice is definitely (Figure 1.11) microtiter plate (also known as 96-well plate.). The science step that first developed this method tried to study the binding of bacteria, not for biofilm. Therefore, it has been observed to be suitable for sessile development. The main method is the growth and reproduction of bacteria in the cavities of microtiter (Djordjevic et al., 2002). The time taken for emptying the wells should be chosen differently. In the next step, to get rid of the planktonic cells, the biomass adhered to the surface of the wells should be washed without staining.

In addition, the biofilm mass can be measured with separation and subsequent coating. Evaluation is made by measuring the biomass in all the wells planted in a 96-well microtiter plate. It is also possible that these parts originate from cells at the bottom of the wells embedded by extracellular polymeric materials (EPS). It would be erroneous to think that this is caused only by the formation of biofilms because it can occur outside of biofilm.

CHAPTER 2

MATERIALS AND METHODS

2.1. Test Microorganisms

Fungal and bacterial strains obtained from Izmir Institute of Technology, Department of Food Engineering and the United States Department of Agriculture Agricultural Research Services Culture Collection (NRRL) were used. Origins of the strains used; *L. monocytogenes* (fresh fruit), *St. aureus* RSSK 01009 (clinical), *L. innocua* NRRL B-33314 (turkey / ham, mad sticks), *Candida albicans* and *Escherichia coli* O157: H7 ATCC700728 (unknown). After the first spread, their growth was monitored by flowing them in nutrient broth (NB). In all experiments in the study, NB was preferred as the medium.

2.2. EO

Commercial pomegranate (*Punica granatum*) seed essential oil (PGEO) and lemon (*Citrus lemonum*) essential oil (CLEO) were purchased from native producer. All the chemicals used were of analytical grade and they were purchased from Sigma Chemical Co. (St. Louis, MO). Different concentrations of EOs were prepared daily. The final concentration of ethanol was not more than 1% in broth. Because dimethyl sulphoxide (DMSO) is toxic and cannot be used in food systems, ethanol was chosen as solvent (Burt *et al*. 2007). Since the chemicals chosen are determined according to their usability in food products, the selection has been made in a narrow environment.

2.3. Culture Media

In the study buffered peptone water (BPW), nutrient broth (NB) sabouraud agar (SA), tryptone soy agar (TSA), PALCAM agar base, MRS (de Man Rogosa and Sharpe), (Merck) were used.

2.4. Cultural Conditions

Cultures were grown in appropriate media and incubation conditions. Then bacterial suspensions were adjusted equivalent to 0.5 McFarland standard by using a Densitometer (DEN-1, HVD Life Sciences, Vienna, Austria) (approximately 7–8 $\log_{10}$/mL) and one more 10-fold dilution was performed in broth medium (Zengin and Baysal, 2015).

2.5. Biofilm Test Surfaces

Stainless steel, glass and polystyrene were chosen because they are the materials most commonly used in food processing equipment and have different physicochemical properties (hydrophobic for polystyrene, hydrophilic for stainless steel).

Stainless steel (SS) coupons preparation The stainless steel (tip 304) coupons were cut into coupons with surfaces of $8 \times 8 \times 0.2$ cm.

Polystyrene (PS) Surface preparation Petri dishes with a diameter of 90 mm and a height of 17 mm were used for the polystyrene material.

Glass Surface preparation Microscope slides were used as glass materials.

2.6. Biofilm Formation

Activated cultures in the study were incubated overnight at 37°C in Tryptic Soy Broth (TSB) with %1 (w / v) glucose added. Subsequently, the twice activated cultures were adjusted to a density of 0.5 McFarland. From these cultures, the densities of which were adjusted, 200 µl was taken and transferred to 96-well microtiter plates. Plates were incubated at 37°C for 6, 12, 24 and 48 hours, respectively. After the incubation period is over, the medium is poured and the plates are washed with sterile PBS solution to remove planktonic cells in the wells and then left to dry at room temperature. After drying, plates were stained with 200 µl of 1% crystal violet solution for 15 minutes, then washed again with PBS solution. Finally, 95% ethanol solution was added to the wells to dissolve the crystal violet and absorbance values were read in the ELISA at 550 nm wavelength. As negative control, TSB was used instead of adding bacterial suspension. Biofilm forming

capacities of strains; OD control> OD test group (non biofilm forming), OD control < OD test group <2∗OD control (weak biofilm), 2∗OD control <OD test group <4∗OD control (moderate biofilm), 4∗OD control < OD test group (strong biofilm) was evaluated according to its formula (Slama et al., 2013; Esteban et al., 2010; Landeta et al., 2013; Guidone et al., 2014). All studies were repeated three times and the results were given based on average values. In order to observe the time-dependent change of the biofilm activities of test microorganism in the study, the study conducted under identical conditions was carried out separately for 24 hours.

2.7. UV-C Irradiation Equipment

UV-C irradiation apparatus consisted of UV-C-emitting monochromatic lamp with peak radiation at 254 nm wavelength. In the chamber of the UV-C device, which was designed from stainless steel the samples were placed in 8 cm distance from lamp. The indoor UV irradiation system used in the study consists of a cabinet (95 cm × 9.75 cm) with a fixed UV-C lamp (Germicidal Lamp, Ultraviolet Inc., NY, USA). For a stable UV-C application, a tray is designed to accommodate samples in a horizontally positioned stainless steel cabinet. Although the distance between the UV lamps and the irradiated sample was adjustable, the project was performed irradiation at a distance of 8 cm. The irradiance I_0 of the lamp was measured by a UV–VIS radiometer placed at the same distance from the UV lamp as the plates.

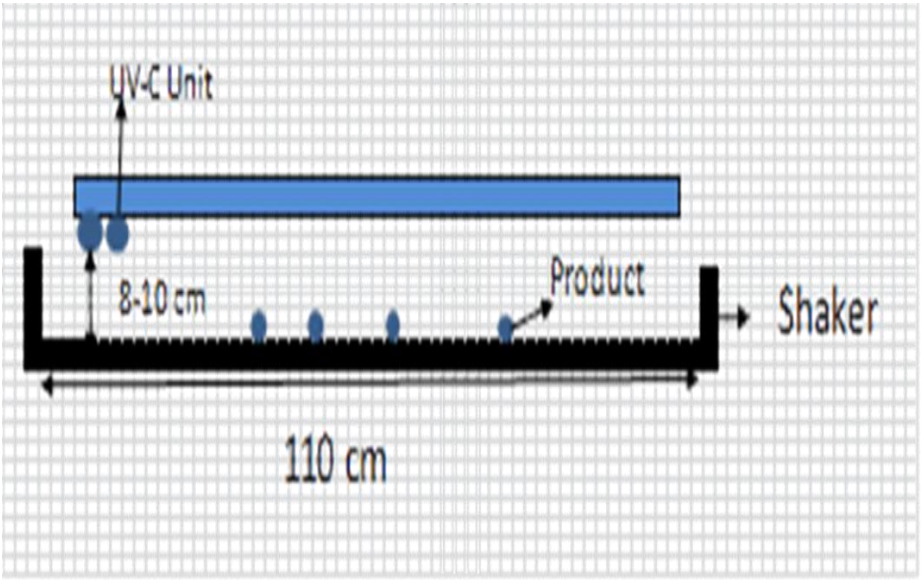


Figure 2.1. UV-C Irradiation Equipment

2.8. UV-C Treatments

Biofilm surfaces were placed on a stainless steel tray, and irradiated with germicidal emitting lamp from upper surfaces 8 cm away from tray. UV-C treatment was applied to obtain radiation treatments between 0-4 kj/m^2 in an enclosed chamber at room temperature with radiation doses using varying exposure times and the UV-C light intensity of radiation as measured by a portable digital radiometer at the surface. The UV-C lamp was turned on for at least 30 min before the experiment to ensure a constant UV-C intensity output and to minimize fluctuations in intensity.

2.9. Essential Oil (EOs) Treatments

2.9.1. Determination antimicrobial effect of EOs

The methods for determining the antimicrobial effects of essential oils are Broth Micro and Macrodilution. A 96-well microtiter plate was used and 180 µL of doubly diluted essential oil and 20 µL of bacterial suspension were added into each well. In the continuation of the broth microdulution, the control wells are formed. These are wells containing culture medium and bacterial suspension, and wells containing only essential oil, that is, not inoculated.

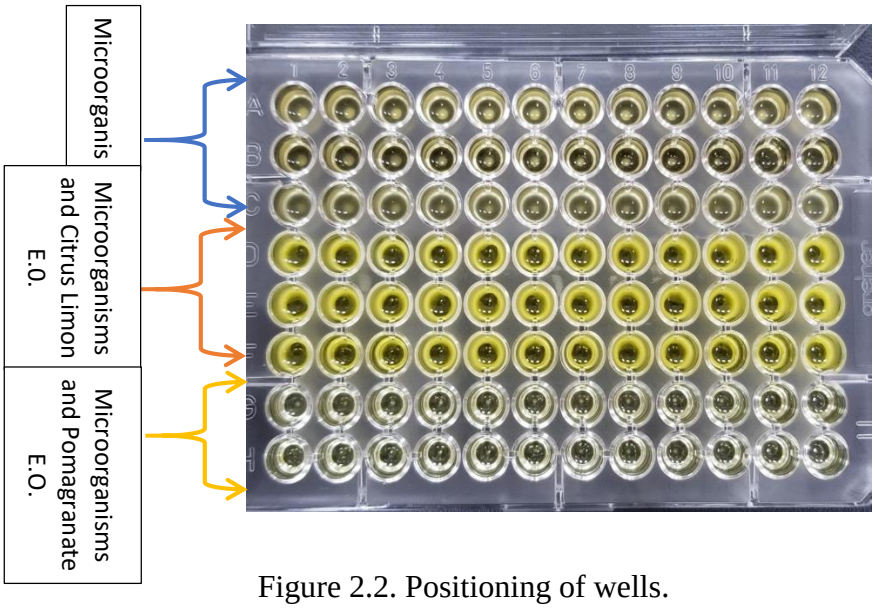


Figure 2.2. Positioning of wells.

Plates were left for a 24 hour incubation. Turbidity after incubation was determined at 600 nm in a microplate reader (Varioskan Flash, Thermo) at 60 minute intervals. Further, smears were made on agar plates after incubation. These smears were made by taking 100 µL of samples from each well for reproduction control. The MIC values of the essential oil were recorded with 96 microplates where vitality was not observed in the wells where the lowest concentrations were determined.

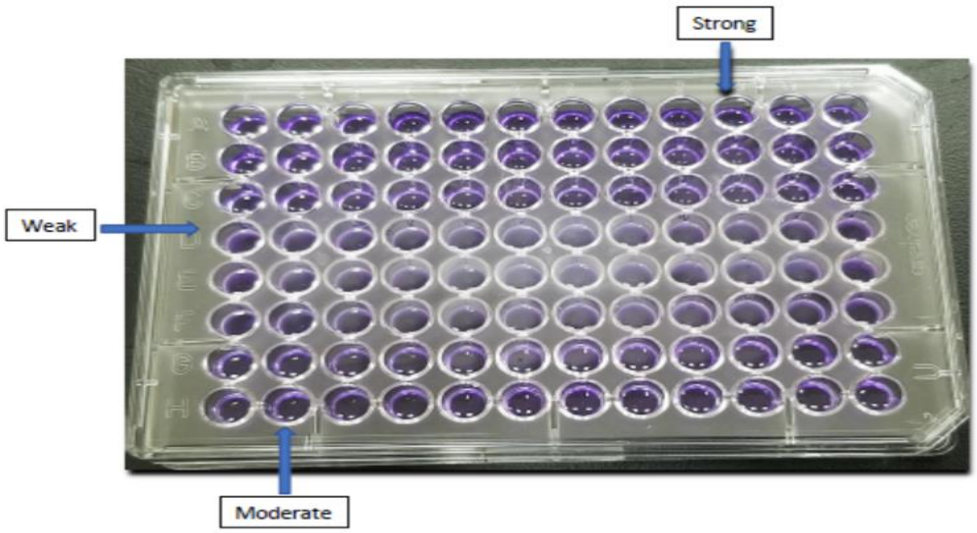


Figure 2.3. Top view of the color difference resulting from the crystal violet

In the other method, the desired essential oil concentrations were adjusted and inoculated into 900 µL of growth medium, that is, into a tube containing 100 µL of bacteria suspension. It was incubated for 24 hours at the desired temperature with shaking. After the incubation period, the sample taken was spread on agar plates.

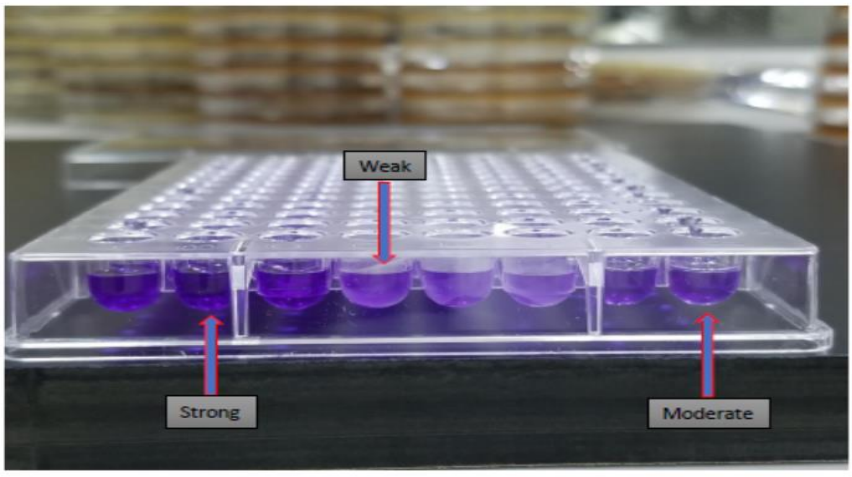


Figure 2.4. Side view of the color difference resulting from the crystal violet

The growth of the colonies was examined after 24-48 hours of incubation of the bacteria spreading to the palaca in the next stage in the broth macro-dilution. Plates without colonies were accepted as MIC values.

2.9.2. Inactivation effect of EOs on bofilm formed on PS, stainless seel and glass surfaces

Pomegranate (*Punica granatum*) seed essential oil (PGEO) and lemon (*Citrus lemonum*) plant essential oil (CLEO) were applied at MIC and sub MIC levels in order to decontaminate *Candida albicans*, *Listeria monocytogenes* and *Staphylococcus aureus single* and *dual* biofilms formed at 20°C in polystyrene microtiter wellplates.

2.10. Enumeration of test Microorganisms after UV-C irradiation and EOs treatments

Biofilms irradiated were enumerated by using swab-vortex method. UV-C treated surfaces were swabbed twice with two separate sterile cotton tips wet with sterile distilled water. Each swab was dipped into 10 ml PW (0.1 %) and vortexed for 1 min.To enumerate surviving population, suspension was then subjected to tenfold serial dilution prior to surface plating.

Each dilution of the samples was surface plated on sabouraud agar and tryptone soy agar for *C. albicans* and *S. aureus*, respectively. *C. albicans* and *S. aureus* counts were determined as the mean of 3 measurements and expressed as the CFU/cm^2. Enumeration of *L. monocytogenes* was carried out by plating aliquots of the 10-fold serially diluted sample onto PALCAM agar base (Oxoid) and incubating the plates at 30°C for 48 h. Triyptic Soy Agar (TSA) for *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Staphylococcus aureus.*

2.11. Statistical Analysis

All the analysis were carried out in triplicate and the experimental results obtained were expressed as means (±) standard deviation. Statistical analysis of the data was made

using analysis of variance (Minitab 16, Minitab Inc., Coventry, UK). Means with a significant difference ($P < 0.05$) were compared using Tukey's test.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. UV-C Treatment Results

Before UV-C treatment the number of *C. albicans S. aureus* and *L. monocytogenes* biofilms formed at 20°C on polystyrene were 3.61 log CFU/cm^2, 3.99 log CFU/cm^2 and 4.27 log CFU/cm^2, respectively.

After 16, 32 and 64 sec UV-C treatment 0.24 log CFU/cm^2, 1.61 log CFU/cm^2, 1.59 log CFU/cm^2 reductions were obtained in the numbers of *C. albicans* biofilms formed at 20°C on polystyrene. In the numbers of *S. aureus* biofilms formed at 20°C on polystyrene 0.99 log CFU/cm^2, 1.9 log CFU/cm^2, 3.91 log CFU/cm^2 reductions were obtained after 16, 32 and 64 sec UV-C treatments, respectively (Fig.3.1).

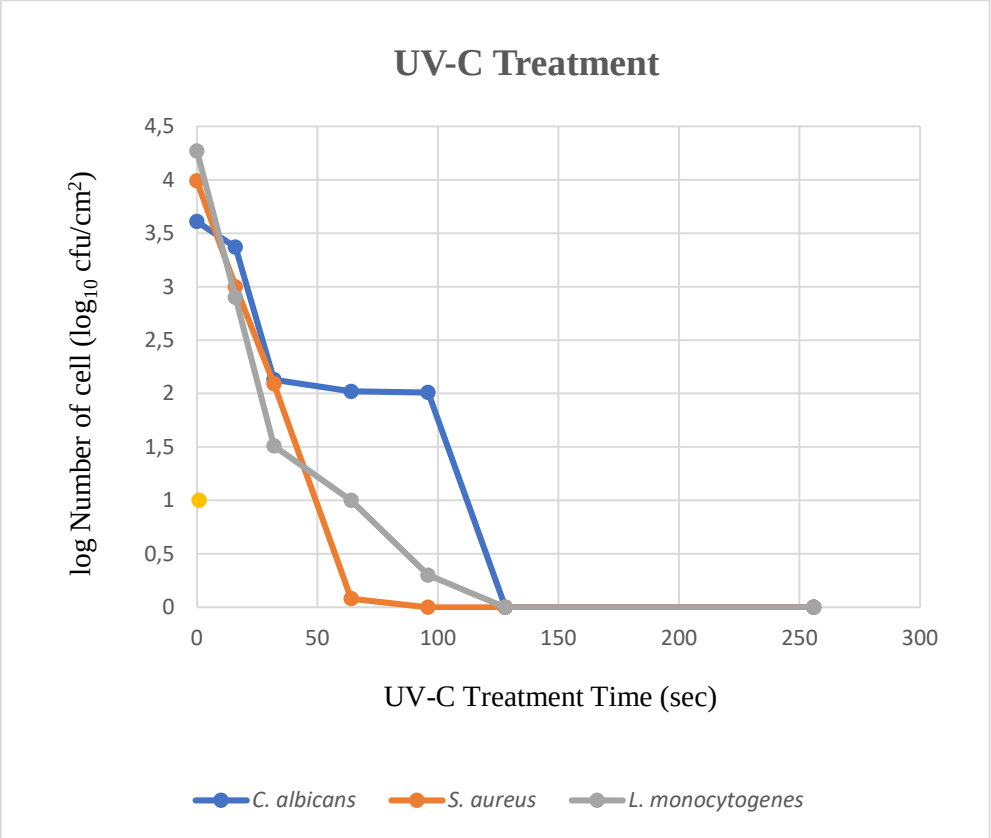


Figure 3.1. Inactivation of *C. albicans*, *S. aureus* and *L. monocytogenes* biofilms formed at 20°C on polystyrene exposed to UV-C.

Reductions in the numbers of *L. monocytogenes* biofilms formed at 20°C on polystyrene after 16, 32 and 64 sec UV-C treatment were determined as 2.25 log CFU/cm^2, 2.76 log CFU/cm^2, 3.27 log CFU/cm^2, respectively (Fig.3.1).

Before UV-C treatment the number of *C. albicans*, *S. aureus* and *L. monocytogenes* biofilms formed at 20°C on stainless steel were 3.82 log CFU/cm^2, 4.25 log CFU/cm^2, 4.72 log CFU/cm^2, respectively.

In the numbers of *C. albicans* biofilms formed at 20°C on stainless steel 0.82 log CFU/cm^2, 0.57 log CFU/cm^2 and 0.69 log CFU/cm^2 reductions were obtained after 16, 32 and 64 sec UV-C treatments, respectively. Reductions in the numbers of *S. aureus* biofilms formed at 20°C on stainless steel after 16, 32 and 64 sec UV-C treatment were determined as 1.25 log CFU/cm^2, 2.36 log CFU/cm^2 and 2.59 log CFU/cm^2. After 16, 32 and 64 sec UV-C treatment 1.82 log CFU/cm^2, 3.64 log CFU/cm^2 and 3.72 log CFU/cm^2 reductions were obtained in the numbers of *L. monocytogenes* biofilms formed at 20°C on stainless steel (Fig. 3.2)

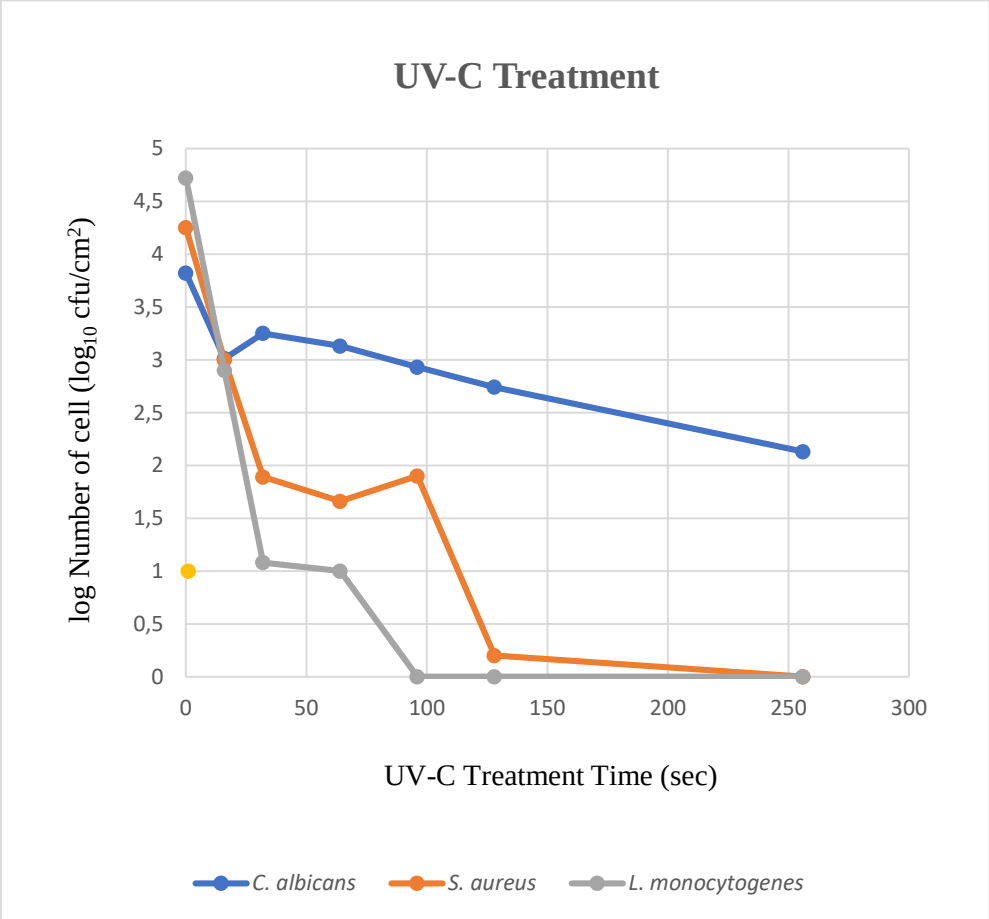


Figure 3.2. Inactivation of *C. albicans*, *S. aureus* and *L. monocytogenes* biofilms formed at 20°C on stainless steel exposed to UV-C

As UV-C dose increased from 0 to 1.16 kJ/cm^2 there was a rapid decrease (1-3.6 log) in the numbers of *S. aureus* and *L. monocytogenes* biofilms formed at 20°C on both polystyrene and stainless steel (Fig. 3.1 and 3.2). However, as seen in Figure 3.2 reductions in the numbers of *C. albicans* biofilms formed at 20°C on polystyrene and stainless steel after 1.16 kJ/cm^2 UV-C treatment were determined as 1.61 log CFU/cm^2 and 0.82 log CFU/cm^2, respectively.

C. albicans, *S. aureus* and *L. monocytogenes* biofilms formed at 20°C on polystyrene were eliminated after 128 sec exposure to UV-C. However, *C. albicans* and *S. aureus* biofilms formed at 20°C on stainless steel were not eliminated after 128 sec UV-C treatment.

In general *C. albicans* biofilm formed at 20°C on stainless steel was found as the most resistant biofilm (Fig. 3.2). The average reduction of *L. monocytogenes* and *S. aureus* biofilms formed at 20°C on both polystyrene and stainless steel surfaces generally increases with increasing dosage of UV-C irradiation (Fig. 3.1 and 3.2).

The reduction of *L. monocytogenes* biofilms formed at 4°C and 20°C on glass surface exposed to UV-C is shown in Table 3.1. After treatment with 3.21 kJ/cm^2 (32 sec) UV-C treatment, *L. monocytogenes* counts of biofilms formed at 4°C and 20°C on glass surface were reduced by 0.79 and 0.75 log CFU/cm^2, respectively. However, for *L. innocua* these values were found as 1.76 and 2.03 log CFU/cm^2.

Table 3.1. Inactivation of *L. monocytogenes* biofilms formed at 4ºC and 20°C on glass surface exposed to UV-C

UV-C Treatment Time (Sec)	***Listeria monocytogenes* (CFU/cm^2)**		***Listeria innocua* (CFU/cm^2)**	
	4°C	**20°C**	**4°C**	**20°C**
0	$1.24x10^3$ (3.09)	$3.42x10^3$ (3.53)	$9.6x10^2$ (2.98)	$7.33x10^3$ (3.87)
16	$2.08x10^1$ (1.32)	$1.45x10^1$ (1.16)	$1.51x10^1$ (1.18)	$2.83x10^1$ (1.45)
32	$1.99x10^2$ (2.30)	$6.01x10^2$ (2.78)	$1.67x10^1$ (1.22)	$6.93x10^1$ (1.84)

The results have demonstrated that *Candida albicans* is more resistant to UV-C than bacteria. Also, *L. monocytogenes* biofilms formed at 4ºC and 20°C on glass surface exposed to UV-C were more sensitive than *L. innocua* biofilms.

3.2. EOs Treatment Results

The present study also aimed to investigate the antimicrobial activity of lemon (*Citrus lemonum*) and pomegranate (*Punica granatum* seed) essential oils and the effects of them when applied to biofilms formed at 4ºC and 20°C on polystyrene exposed to UV-C.

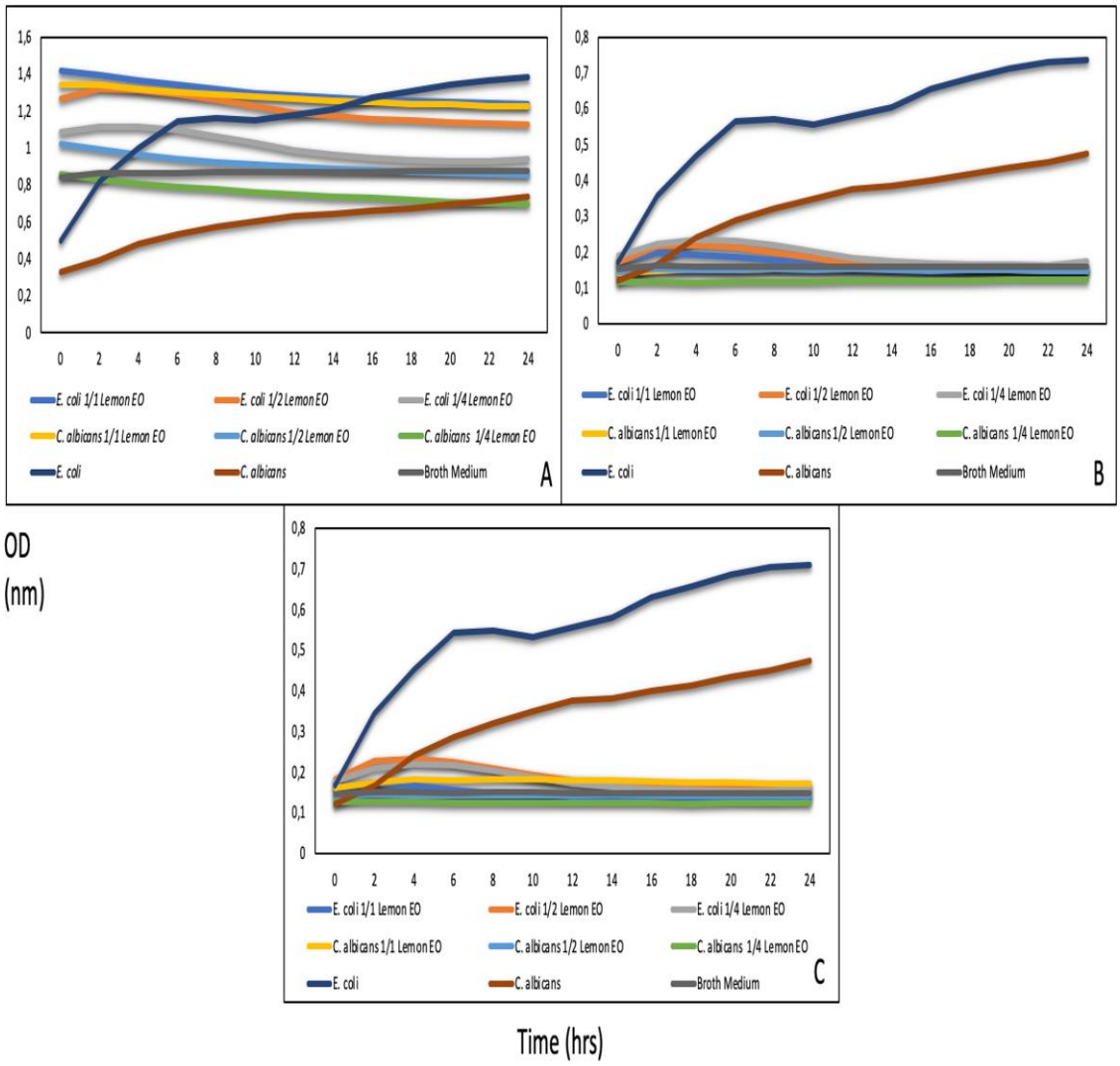


Figure 3.3. The growth and inhibition of *E. coli* O157:H7 *and C. albicans* on exposure to to lemon (*Citrus lemonum*) essential oil with OD values at 405 nm (A), 600 nm (B), 620 nm (C).

Antimicrobial activity was examined against *S. aureus*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, *L. lactis* and *C. albicans*. Lemon (*Citrus lemonum*) essential oil (CLEO) was effective on the bacteria with different concentrations. The findings represented that lemon (*Citrus lemonum*) essential oil restricted the growth of *C. albicans* and *E. coli* O157:H7 (Fig.3.3). The CLEO did not have a significant inhibition effect on the *Lactococcus lactis* (Fig.3.4).

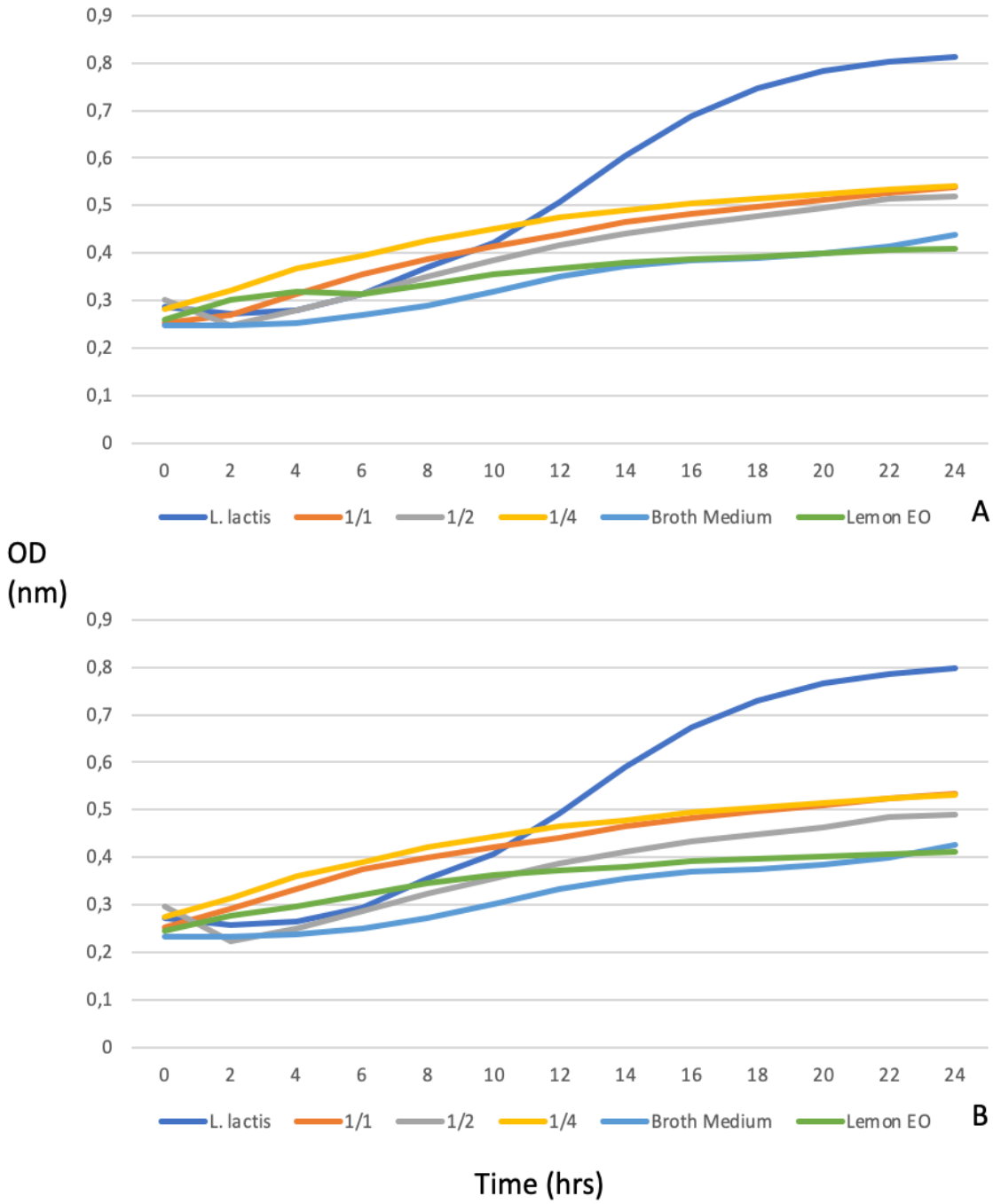
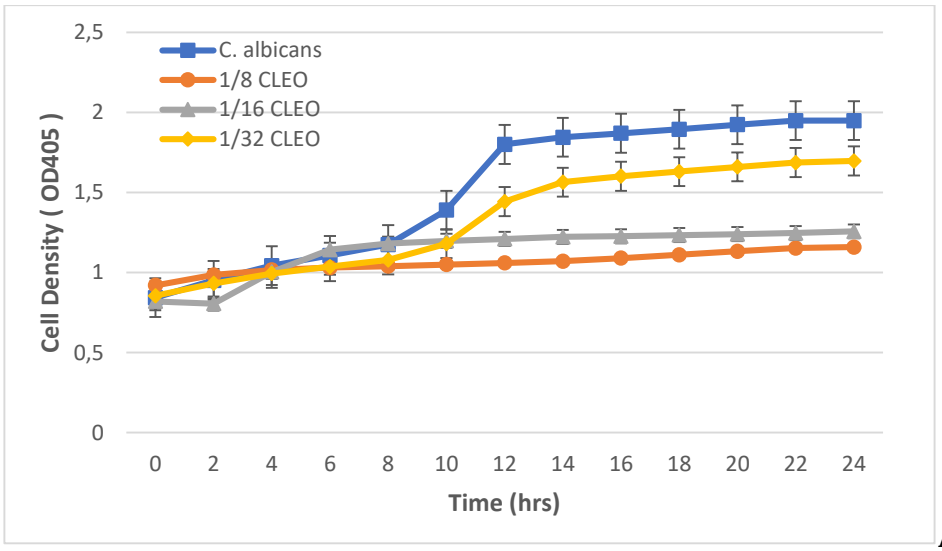


Figure 3.4. The growth and inhibition of *L. lactis* on exposure to to lemon (*Citrus lemonum*) essential oil with OD values at 600 nm (A), 620 nm (B).



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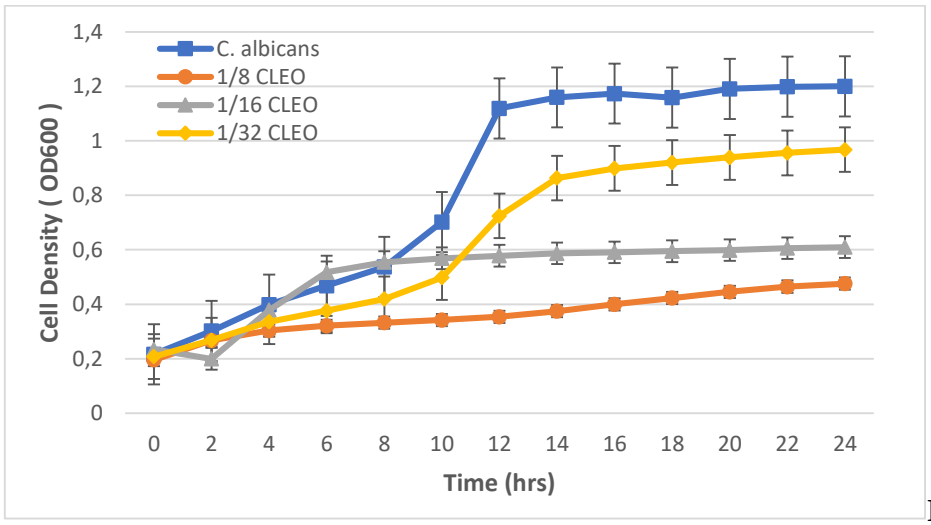

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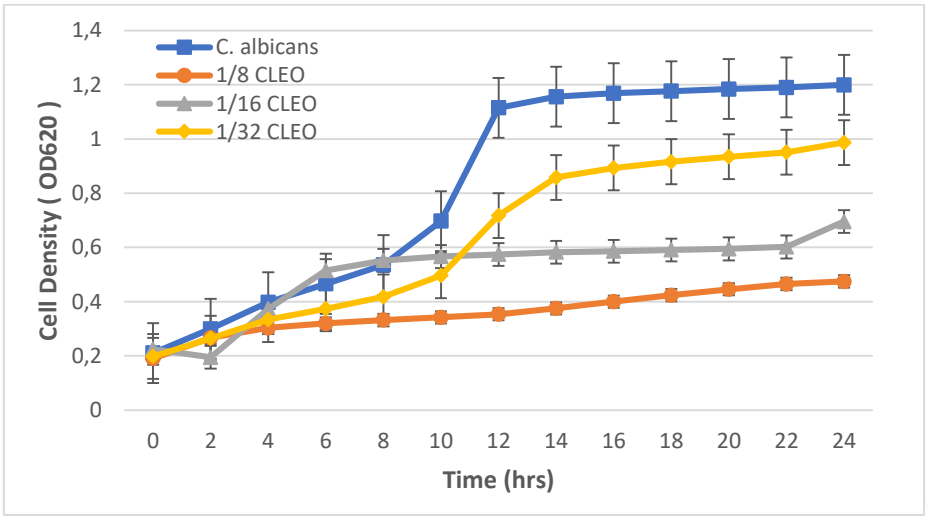

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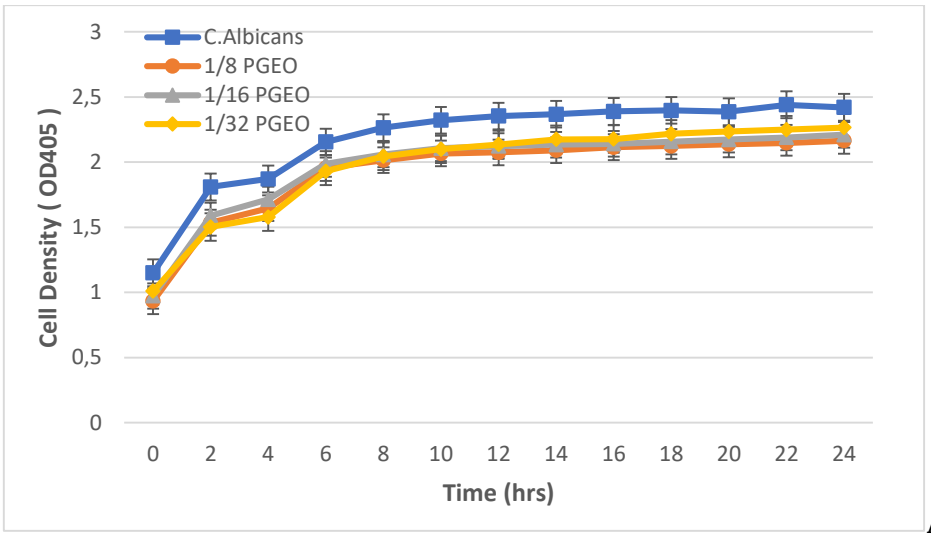
Figure 3.5. The growth and inhibition of *C. albicans* on exposure to lemon (*Citrus lemonum*) essential oil with OD values at 405 nm (A), 600 nm (B), 620 nm (C).



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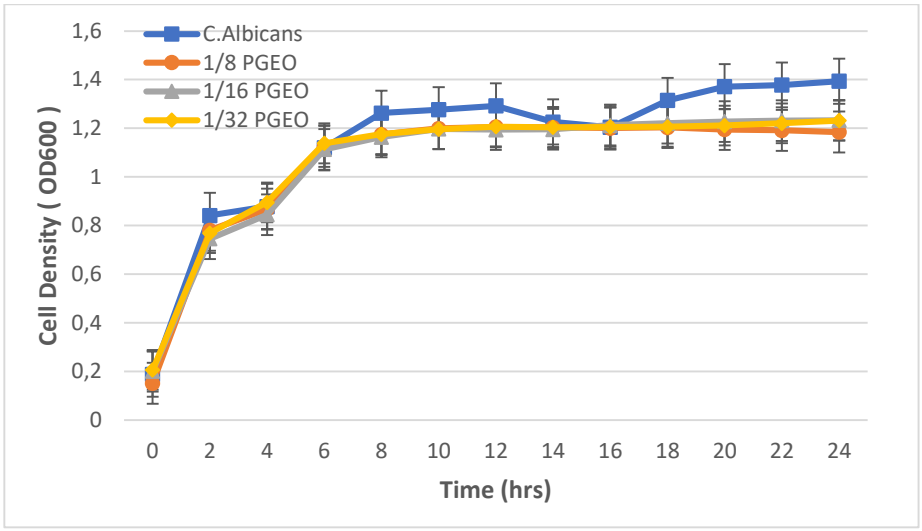

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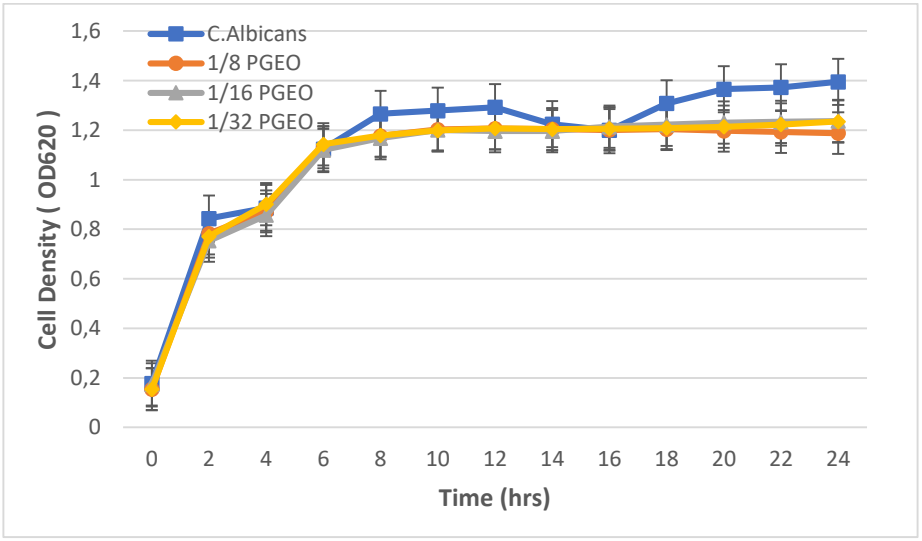
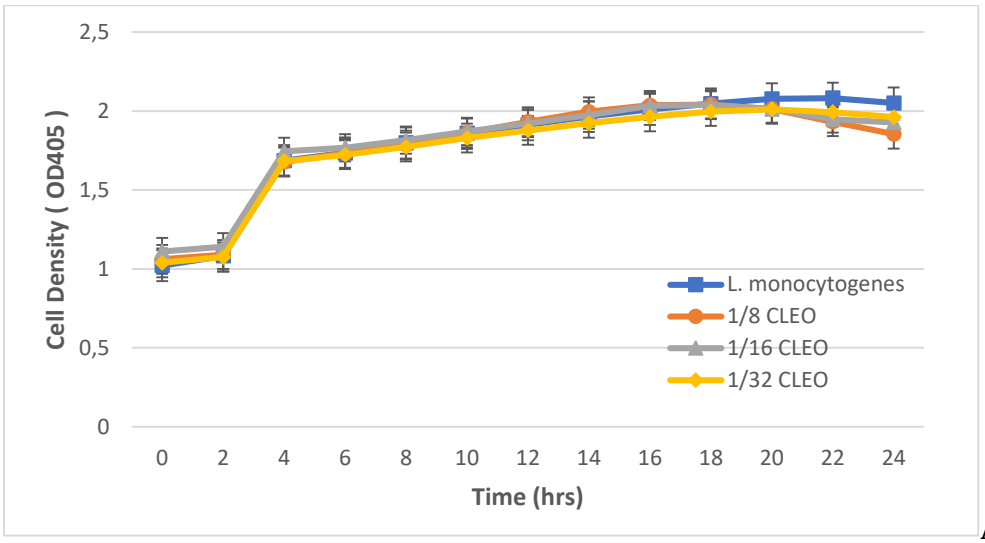


C

Figure 3.6. The growth and inhibition of *C. albicans* on exposure to PGEO (*Punica granatum* seed essential oil) with OD values at 405 nm (A), 600 nm (B), 620 nm (C).



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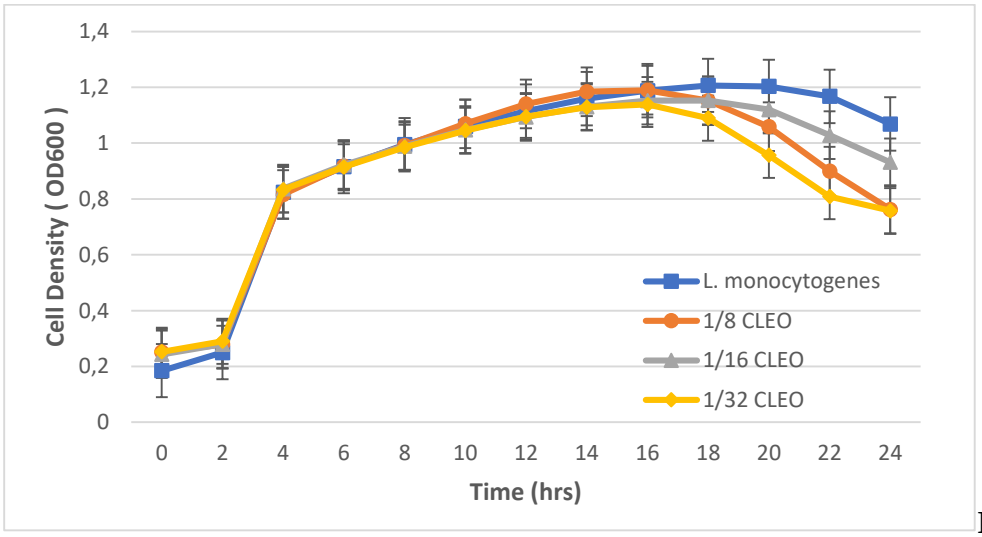

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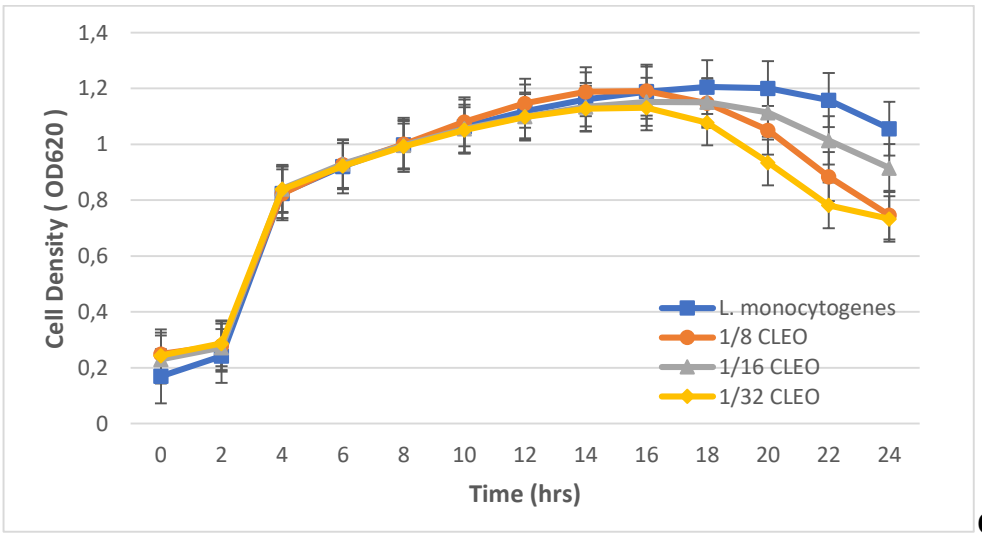

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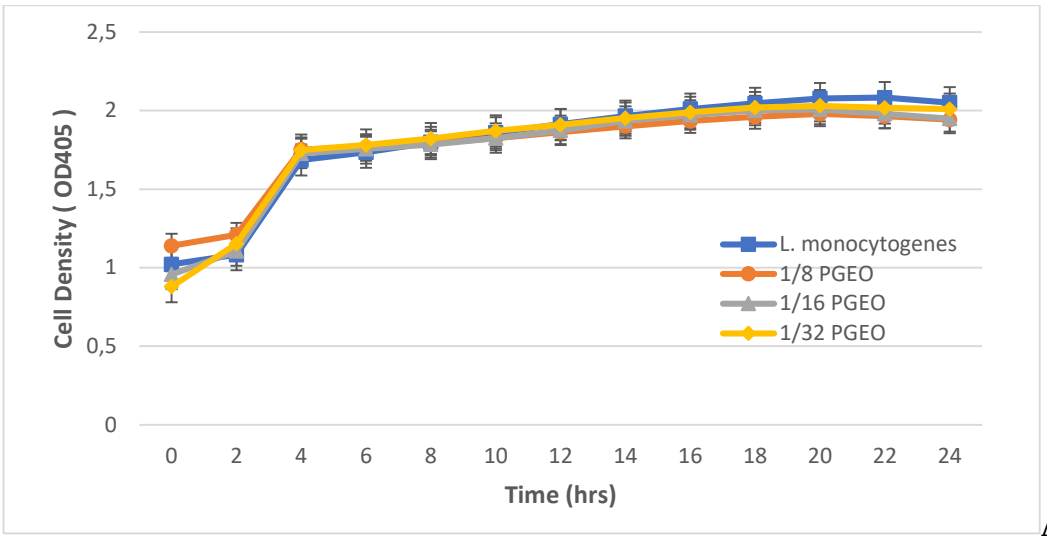
Figure 3.7. The growth and inhibition of *Listeria monocytogenes* on exposure to lemon (*Citrus lemonum*) essential oil with OD values at 405 nm (A), 600 nm (B), 620 nm (C).



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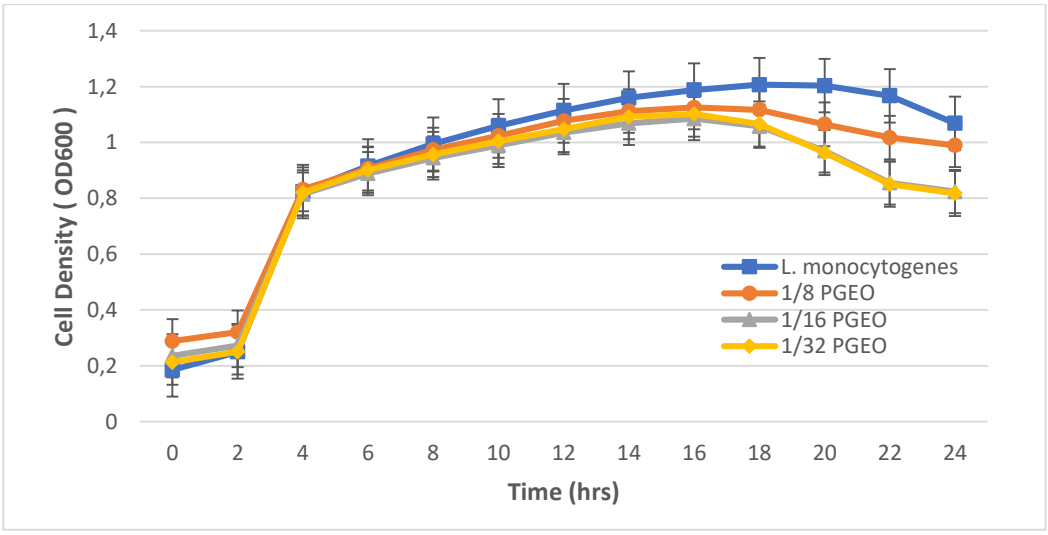

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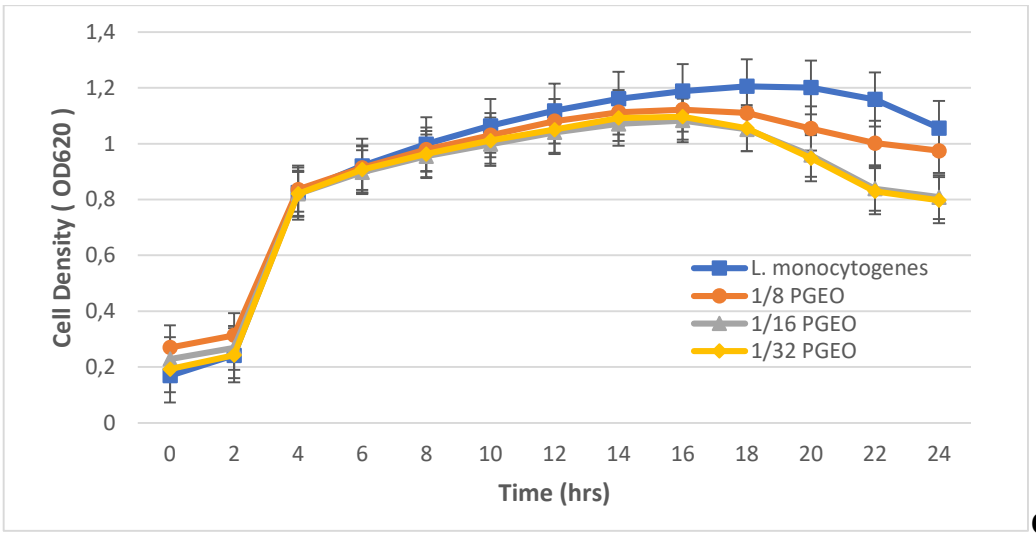

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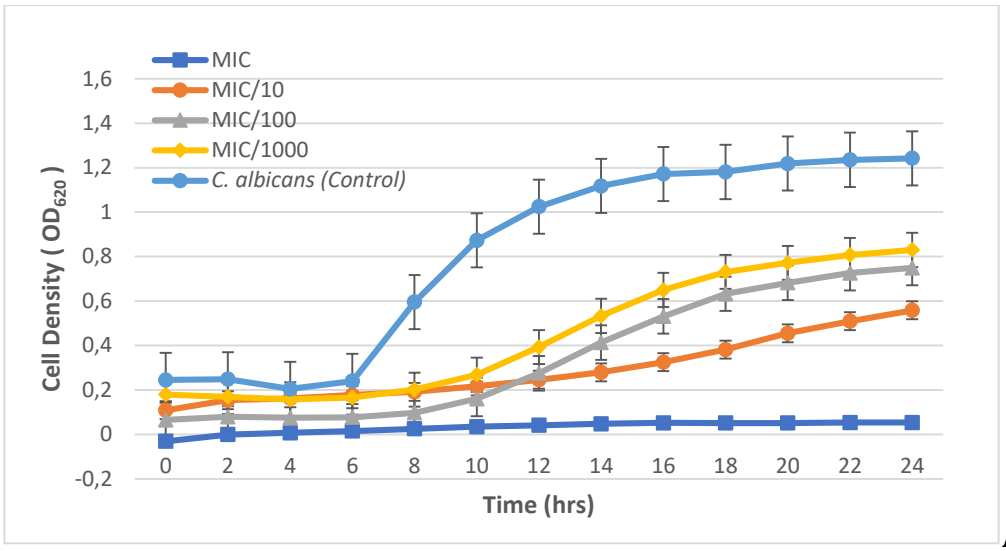
Figure 3.8. The growth and inhibition of *L. monocytogenes* on exposure to pomegranate seed essential oil (PGEO) with OD values at 405 nm (A), 600 nm (B), and 620 nm (C).



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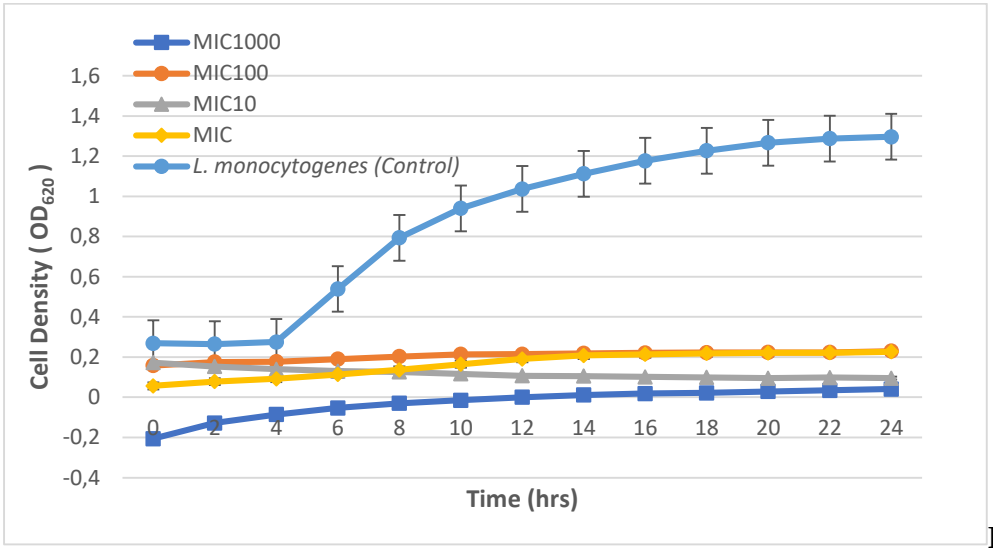

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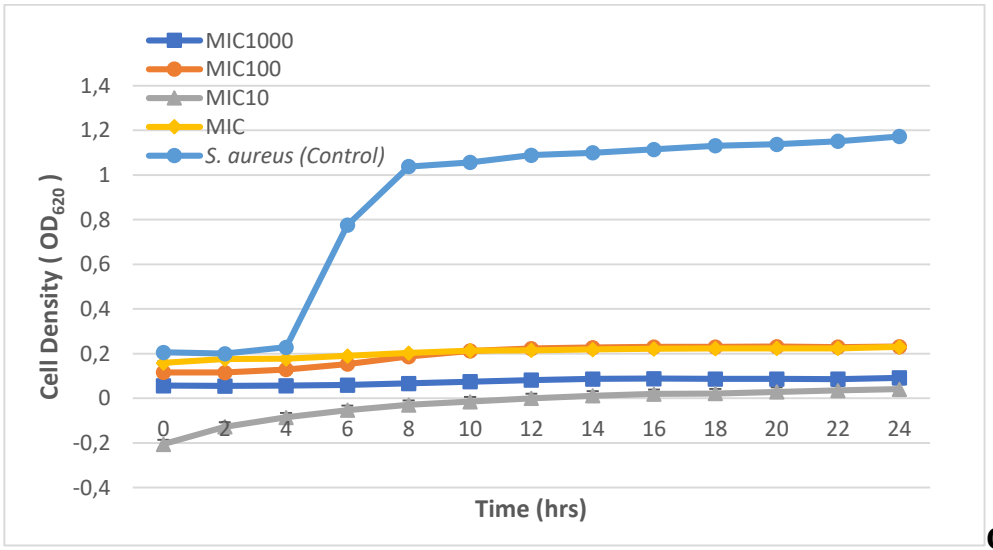

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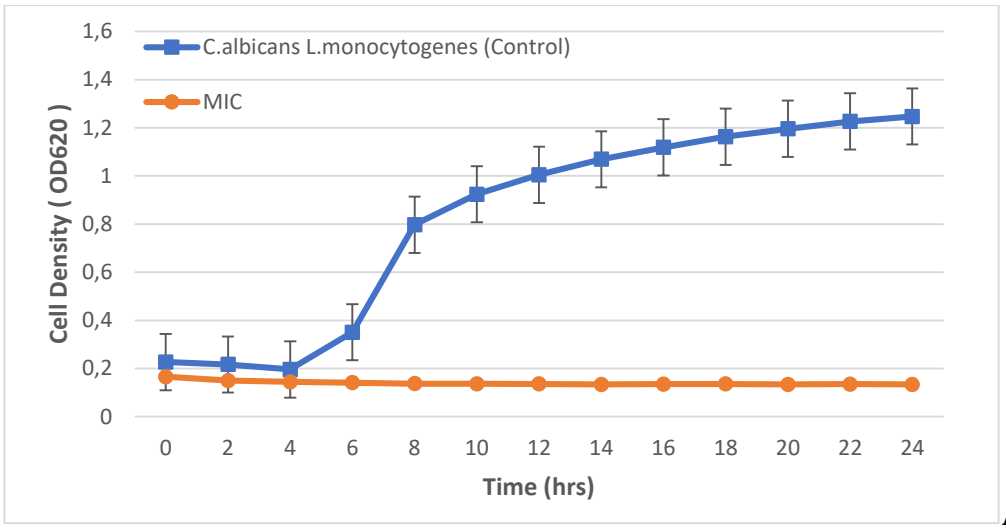
Figure 3.9. The growth and inhibition of *C. albicans* (A), *L. monocytogenes* (B), and *S. aureus* (C) on exposure to lemon (*Citrus lemonum*) essential oil.



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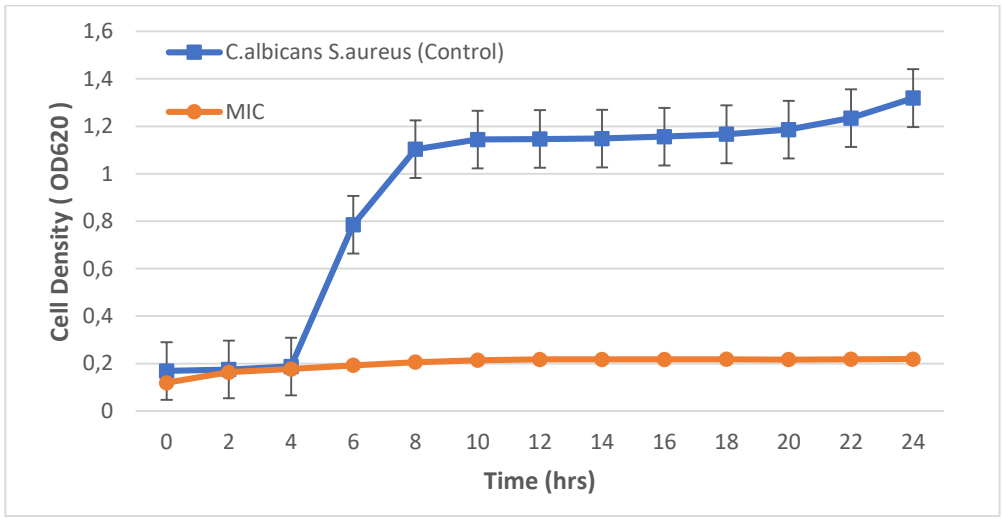

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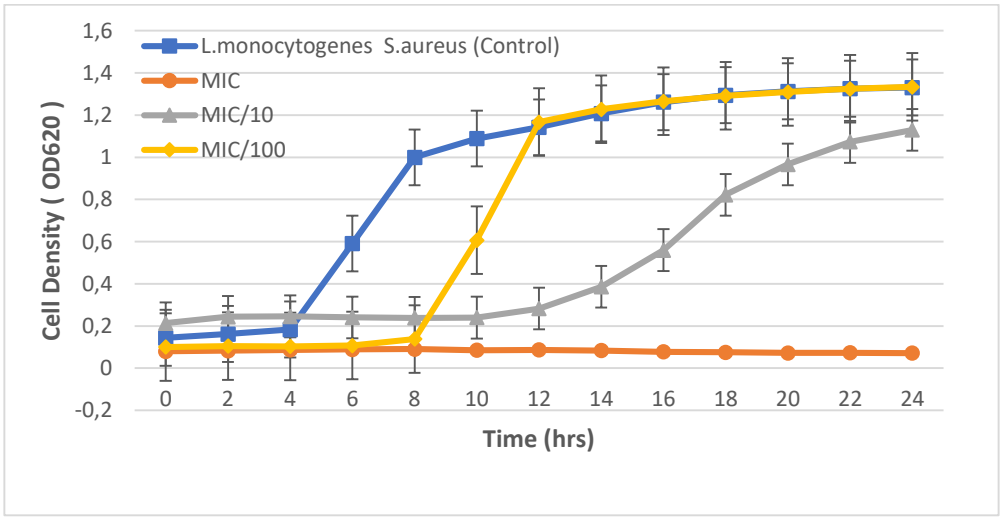

C

Figure 3.10. The growth and inhibition of mix culture *C. albicans* and *L.monocytogenes* (A), *C. albicans* and *S. aureus* (B), *L. monocytogenes* and *S. aureus* (C) on exposure to lemon (*Citrus lemonum*) essential oil. essential oil.

The minimum inhibitory concentrations (MICs) of lemon (*Citrus lemonum*) essential oil (CLEO) prevented biofilm formation of *C. albicans* (A), *L. monocytogenes* (B), and *S. aureus* (C). Also, 1/10 MIC, 1/100 MIC and 1/1000 MIC of CLEO prevented biofilm formation of *L. monocytogenes* and *S. aureus*. It was observed that biofilm formation decreased with increased concentration. 1/10 MIC, 1/100 MIC and 1/1000 MIC of CLEO inhibited biofilm formation of *C. albicans* for 10 h.

Table 3.2. MICs of pomegranate (*Punica granatum*) seed essential oil (PGEO) and lemon (*Citrus lemonum*) essential oil (CLEO) against test microorganisms.

Test Microorganisms	**MICs (mg/ml)**	
	PGEO	CLEO
Staphylococcus aureus	ND	0,10350
Escherichia coli O157:H7	ND	ND
Listeria monocytogenes	ND	0,10350
Candida albicans	ND	0,1863l

ND: Not determined.

The antimicrobial effect of CLEO was assessed by determining the MIC against selected food poisoning pathogens. CLEO inhibited the growth of *C. albicans*, *L. monocytogenes* and *S. aureus* at 186 µg/ml, 103.5 µg/ml and 103.5 µg/ml, respectively.

The inhibitory effect of CLEO on biofilms formed by food poisoning pathogens is shown in Fig. 3.9. Biofilm inhibition effect represented inhibitory effects in the initial stage of biofilm formation. In the crystal violet staining assay, *C. albicans*, *L. monocytogenes* and *S. aureus* biofilms were significantly inhibited at concentrations above MIC, 1/1000 × MIC (0.1035 µg/ml) and 1/1000 × MIC (0.1035 µg/ml) , respectively. Interestingly, CLEO inhibited the formation of biofilms at a concentration below the MIC.

Citral that is main component of the CLEO has been reported to exhibit antimicrobial activity against pathogenic and food-spoilage bacteria such as *E. coli* O157:H7, *Salmonella*.

CHAPTER 4

CONCLUSIONS

Results of this study demonstrated that lemon essential oil is an effective against both planktonic (free floating) cells and sessile (attached) cells.

It was found to be more effective in inhibiting growth and biofilm formation of bacteria (*S. aureus, L. monocytogenes, E. coli*) than yeast *Candida albicans*. UV-C light treatment was also effective for inactivation of biofilm formed on surface of polystrene, stainless steel and glass. Both of the used treatments can be found an application in the food industry as sanitizer, disinfectant for food contact an non food contact surfaces.Also it is possible to use both approach together as hurdle concept for a milder process application.

The effect of various essantiol oils and their indivudual constituents on biofilms formed by *L. monocytogenes, S. aureus, E. coli* and *C. albicans* has been investigated previously.

However, to our knowledge, this the first report of the anti-listerial, anti bacterial (*S. aureus, E. coli*) anti-fungal (*C. albicans*) and antibiofilm effects of pomegranate seed and lemon essential oils, at different temperatures, on different surface types (polystyrene, stainless steel and glass) by comparison UV-C light.

Cells in a biofilm have been observed to be more resistant to ; heat, drying, acidic conditions, salinity, antimicrobials and food preservatives compared to their planktonic forms. Therefore, better sanitizing and disinfecting strategies are required to deal with this problem. In addition the emergence of antibiotic resistant strans and consumer attitudes towards consuption of less chemicals used food items encouraged devolopment of natural antimicrobial agents, therefore we studied UV-C and two natural essantial oil.

In addition, chemical components other than the active ingredient terpene found in lemon oil can be examined in future research, and their mechanism of action can work and reveal an important advantage against the current bacterial resistance for different antimicrobials.

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