

Full Length Research Paper

# Scanning electron microscopy of biofilm formation by *Staphylococcus aureus* on stainless steel and polypropylene surfaces

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Attachment of bacteria to food processing equipment surfaces can lead to product contamination, spoilage, and surface destruction. Most of bacteria in their habitat live in communities of different degrees of complexity, in general composing biofilms. Once installed the biofilm, the resistance of these sessile organisms increases, making it difficult thus the action of sanitizers. This multidisciplinary study led to the description regarding the effect of antimicrobial solutions of essential oils of *Syzygium aromaticum* and *Thymus vulgaris* and their combination on biofilm formed by *Staphylococcus aureus* ATCC 25923 on AISI 304 stainless steel and polypropylene surfaces and evaluate the possible effects of these oils on *Staphylococcus aureus* cells after treatment with sanitizing solutions based on these natural compounds. All sanitizing solutions showed antibacterial potential, being effective in reducing bacterial biofilms on these surfaces. The solution containing the combination of essential oils was the most efficient by reducing 7.38 and 6.58 Log CFU.cm<sup>-2</sup> of cells adhered on the surfaces of AISI 304 stainless steel and polypropylene, respectively, after 5 min of contact.

**Key words:** Antimicrobial effect, microorganism, *Syzygium aromaticum*, *Thymus vulgaris*.

## INTRODUCTION

Biofilms are surface-attached microbial communities found ubiquitously in almost any kind of environment. In the process of building a biofilm, bacterial cells form

aggregated microcolonies surrounded by thick extracellular polymeric substances (EPS) that include nucleic acids, proteins, lipids and polysaccharides.

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The EPS serves as the structural scaffold and offers protective shielding of biofilm bacteria, which dramatically increases bacterial resistance to sanitizers and antimicrobial agents. Biofilm formation is a dynamic process which in a sequential manner involves attachment, microcolony formation and maturation as well as dispersal (Tan et al., 2014).

Bacteria communicate via a phenomenon termed quorum-sensing (QS) in which they secrete and detect chemical signal molecules in their surrounding environment (Fuqua et al., 1994). Quorum-sensing allows a bacterial community to activate a coordinated cellular response such as virulence factor production, biofilm development and shielding (Alhede et al., 2009). Quorum-sensing has been implicated in food spoilage (Skandamis et al., 2012). As such, QS is a potential target for the prevention/eradication of biofilms in the food industry.

In the food industry, biofilm formation leads to serious health and economic losses due to food contamination and equipment damage. Biofilms can develop the most varied surfaces, and those most used in food production plants is the AISI 304 stainless steel (American Iron and Steel Institute) and polypropylene. The microorganisms present in the biofilm catalyze chemical and biological reactions causing corrosion of metal pipes and tanks, reducing heat transfer due to the thickness of the biofilm, among others (Shafahi and Vafai, 2009).

Among these microorganisms, *Escherichia coli* and *Staphylococcus aureus* are usually found in nature, their presence in food being the consequence of the often precarious sanitary conditions of food production, due to contaminated handling or surfaces. In the food industry, biofilm formation leads to serious hygiene problems and economic losses, mainly due to food contamination, spoilage and damage to equipment. Once established, biofilms act as points of constant contamination, releasing pathogenic and / or spoilage microorganisms (Boari et al., 2009).

Surveys have been documented involving food contact surfaces and various microorganisms, such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *E. coli* O157: H7, *S. aureus* and *Pseudomonas aeruginosa*, among others (Shi and Zhu, 2009).

In the quest for better understanding of the biofilm formation process on different surfaces, the search for, and research on sanitizing agents and antimicrobial alternatives should be generated. In this context, it has been observed that the essential oils found in condiment plant extracts have antibacterial, antifungal and antioxidant properties, which has aroused the interest of food industries (Di Pasqua et al., 2007; Kalemba and Kunicka, 2003; Lambert et al., 2001).

The inhibitory effect of these oils on microorganisms is an alternative to reduce the use of chemical additives in food and for the formulation of new sanitizing agents. Various studies have shown that essential oils extracted

from leaves and different parts of plant species have high antimicrobial activity (Bakkali et al., 2008; Gill and Holley, 2006; Burt, 2004; Lambert et al., 2001).

Given the above, the objective of this study was to evaluate the action of sanitizing solutions formulated with essential oils of clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) and their combinations, on bacterial biofilms formed by *Staphylococcus aureus* on AISI 304 stainless steel and polypropylene surface and evaluate the possible effects of these oils on *S. aureus* cells after treatment with sanitizing solutions based on these natural compounds.

## MATERIALS AND METHODS

Microbiological analyzes were performed in the Food Microbiology Laboratory, Food Science Department, Universidade Federal de Lavras (UFLA), MG. The electron micrographs of the structure of mature biofilms and sanitized were obtained from scanning electron microscopy, from the Laboratory of Electron Microscopy and Ultrastructural Analysis, Plant Pathology Department, Universidade Federal de Lavras (UFLA), MG.

### Microorganism used, standardization, inoculum preparation and storage

The microorganism used in the development of this study was *S. aureus* ATCC 25923. The culture of *S. aureus* was maintained at -18°C in microcentrifuge tubes with freezing medium [glycerol (150 mL), peptone (5 g), yeast extract (3 g), NaCl (5 g), H<sub>2</sub>O (1.000 mL), pH 7.2 ± 7.4]. During the experiment, subcultures were made for the maintenance of cultures. Aliquots were transferred to the microcentrifuge tubes containing tryptic soy broth (TSB) and incubated at 37°C/24 h. After culturing, 1 mL of the culture was dispensed into sterile microcentrifuge tubes and centrifuged at 6,000 x g for 8 min in a microcentrifuge. After removing the supernatant, the content was again coated with freezing medium and stored at -18°C.

For reactivation and use of the strain, 10 µL of the culture was inoculated in tubes containing 3 mL of TSB and incubated at 37°C/24 h. After incubation, 20 µL of the inoculum was removed and transferred to 200 mL of TSB. The number of cells per millilitre was quantified using standard curve and the growth monitored by spectrophotometry at 600 nm and then counting on plates. The bacterial culture was standardized to a concentration of 10<sup>8</sup> CFU/mL.

### Experimental model of biofilm formation

#### Preparation and cleaning of coupons

The bacterial adhesion was conducted on AISI 304 stainless steel and polypropylene coupons with 1 mm thickness and dimensions of 10x20 mm.

The AISI 304 stainless steel coupons were cleaned individually with 100% acetone, submerged in detergent, rinsed with sterile distilled water, dried and cleaned with 70% ethanol (v/v). After cleaning, the coupons were again washed with sterile distilled water, dried for 2 h in oven at 60°C and autoclaved at 121°C/15 min (Rossoni and Gaylarde, 2000). As for the polypropylene coupons, they were initially immersed in a solution of commercial 0.3% peracetic acid for 30 min under stirring at 50 rpm at 50°C. They were then soaked in sterile distilled water at 80°C for 5 min and at

**Table 1.** Composition of the sanitizing solutions.

Sanitizing solution	Composition (mL)		
	Saline solution	Ethanol	Essential oil
Control	8.00	2.00	0.00
<i>S. aromaticum</i>	8.00	1.99	0.01
<i>T. vulgaris</i>	8.00	1.99	0.01
Combination*	8.00	1.99	0.005

\*Combination of oils of *S. aromaticum* and *T. vulgaris* at a 1:1 proportion.

room temperature for 1 min under agitation of 50 rpm. The coupons were dried at 40°C for 2 h and autoclaved for 15 min at 115°C (Oulahal et al., 2008).

#### Adhesion of bacterial cells to surfaces

In two Petri dishes (140 mm diameter) 45 AISI 304 stainless steel coupons and 80 mL TSB were added and inoculated with  $10^8$  CFU/mL of culture. In two other Petri dishes the same procedures were employed, however, with 45 polypropylene coupons, with the aim of promoting the formation of biofilms on these surfaces. The plates were incubated at 37°C with orbital agitation of 50 rpm. At 48-h intervals, the coupons were collected, washed with peptone water (0.1% w/v) five times and immersed in TSB contained in sterile plates. This procedure was performed five times in order to complete the formation of the biofilm after 10 days of incubation (Joseph et al., 2001).

#### Enumeration of adhered cells

For enumerating the adherent cells, one AISI 304 stainless steel and one polypropylene coupon was removed from each Petri dish every two days of incubation. These were washed with peptone water (0.1% w/v) five times to remove planktonic cells and the sessile cells were collected using a standard sterile cotton swab. The swabs were transferred to tubes containing peptone water (0.1% w/v) and then agitated in a vortex for 2 min. After this procedure, serial dilution was carried out in which 0.1 mL aliquots were plated and the number of viable cells quantified in Tryptic Soy Agar (TSA), using the surface seeding technique. The plates were incubated at 37°C/24 h, conducting a standard plate count at the end of this period, results expressed in CFU/cm<sup>2</sup> (Joseph et al., 2001).

#### Obtention of essential oils

The essential oils of *S. aromaticum* and *T. vulgaris* were purchased through the Ferquima Ind. e Com. Ltda Company (Vargem Grande Paulista, São Paulo, Brazil); their physical and chemical parameters being described by the supplier, which produces and sells essential oils on an industrial scale.

#### Preparation of sanitizing solutions

To perform the sessile cell sensitivity test, four sanitizing solutions

were formulated containing saline (NaCl 0.85% w/v), ethanol (p.a.95% v/v) and essential oil as shown in Table 1.

All sanitizing solutions contained a total volume of 10 mL and the amount of essential oils used in the formulation of the sanitizing solutions was defined from the Minimum Inhibitory Concentration (MIC) test results previously performed by disk diffusion technique (NCCLS, 2000 with modifications).

#### Treatment of biofilms with sanitizing solutions containing essential oils at different contact times

On the tenth day of cultivation, polypropylene and steel coupons were taken from each Petri dish, rinsed in 0.1% peptone water (v/v) five times to eliminate non-adherent cells, and immersed in the above sanitizing solutions for durations of 5 and 10 min at room temperature. After the sanitizing, the coupons were rubbed with standardized sterile swabs. The swabs were transferred to tubes containing 0.1% peptone water (v/v) and then agitated in a vortex for 2 min. After this procedure, serial dilution was conducted, 0.1 mL aliquots were plated and the number of viable cells determined in TSA medium, using the surface seeding technique. The plates were incubated at 37°C for approximately 24 h, conducting the standard plate count at the end of this period with, results expressed in CFU/cm<sup>2</sup> (Joseph et al., 2001; with adaptations).

#### View the surfaces of coupons by scanning electron microscopy

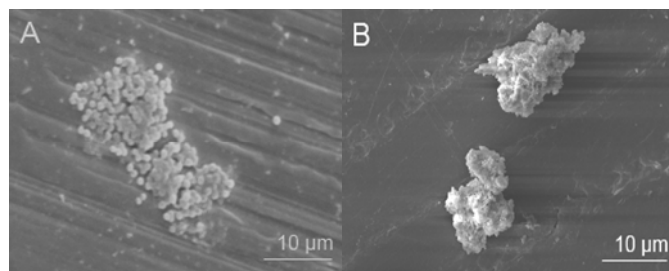
The coupons of stainless steel and polypropylene containing biofilm and sanitizers were immersed in fixative solution (modified Karnovsky), pH 7.2, for 48 h. The coupons were cleaned by soaking for 10 mins in cacodylate buffer, and this procedure was repeated three times and posteriorly were fixed in 1% osmium tetroxide in water for three hours. After this process, were washed three times in distilled water and dehydrated in ethanol gradient (25, 50, 70, 90, 95%, 10 min and 100% three times for 10 min). Next was the drying of this material in a critical point apparatus (Bal-Tec CPD 030), mounting stubs and covered with a thin gold layer (Bal-Tec SCD sputter 050). Electron micrographs of the bacteria adhered to the surfaces of stainless steel and polypropylene were obtained before and after treatment with sanitizing solutions, and a scanning electron microscope Leo 040 Evo (Alves, 2004).

#### Experimental design and statistical analysis

A completely randomized design (CRD) was used in a 2 x 5 factorial outline with 3 replicates, the surface factor having 2 levels: stainless steel and polypropylene, the time factor with five quantitative levels: 48, 96, 144, 192 and 240 h. The enumeration of adhered cells on the stainless steel and polypropylene coupons after treatment with these sanitizing solutions at different contact times, used the CRD in a factorial scheme (4 x 2 x 2) with three replicates with the factor agents at four qualitative levels: control, *S. aromaticum*, *T. vulgaris* and combination, the factor surfaces with two qualitative levels: stainless steel and polypropylene, and the time factor with two quantitative levels: 5 and 10 min. Statistical analyzes were performed using the SISVAR (Ferreira, 2003) and R Development Core Team programs (R Development Core Team, 2004).

**Table 2.** Identification and quantification of chemical constituents

Peak	Constituent ID	%
<b><i>Syzygium aromaticum</i></b>		
1	Eugenol	92.3
2	Acetileugenol	0.2
3	Caryophyllene	5.5
4	$\alpha$ -Humulene	0.9
<b><i>Thymus vulgaris</i></b>		
1	$\alpha$ -Pinene	2.2
2	Camphene	0.8
3	Myrcene	1.4
4	$\rho$ -Cymene	26.8
5	1.8-Cineol	1.3
6	$\gamma$ -Terpinene	6.0
7	Linalool	5.2
8	Camphor	1.5
9	Borneol	0.9
10	Thymol	47.3
11	Carvacrol	3.1
12	$\beta$ -Caryophyllene	0.8

**Figure 1.** Scanning electron micrographs demonstrating adherence of *Staphylococcus aureus* after 240 h of biofilm formation, the surfaces stainless steel of AISI 304 (A) and polypropylene (B).

## RESULTS AND DISCUSSION

Table 2 shows the counts of sessile cells adhered to the surfaces of the AISI 304 stainless steel and polypropylene coupons during the biofilm formation process.

The adhesion of bacterial cells depends on factors such as physiology and cell morphology and physico-chemical properties of the contact surface. Gram negative microorganisms have greater ease of adhesion on surfaces when compared to Gram positive, as they have pili, flagella and fimbriae, as well as an outer membrane. Microorganisms electrically charged with

negative charges have more difficulty to link directly to surfaces. The participation of a conditioning film formed by various compounds and molecules from the aqueous phase, will be decisive.

It can be observed that the microbial cells adhered similarly to both surfaces up to 192 h and differed significantly only at the end of the biofilm formation process that is at 240 h. The electron micrographs in Figure 1 illustrate cell adhesion of *S. aureus* on surfaces stainless steel of AISI 304 and polypropylene.

The adhesion of bacterial cells depends on factors such as physiology and cell morphology and physico-chemical properties of the contact surface.

The ability of *S. aureus* to adhere to solid surfaces producing compounds by multilayered cells embedded in a exopolysaccharide matrix is considered one of the relevant aspects of the epidemiology of this bacterium (Cucarella et al., 2001; Flach and Karnopp, 2005). This organization is extremely beneficial to all species of microorganisms, because it provides protection against adversity such as dehydration, colonization by bacteriophages and antimicrobial resistance (Gilbert et al., 2003).

By the phenomenon known as passivation, chromium, due to its high affinity for oxygen, tends to combine with it, forming a thin layer of chromium oxide with an approximate thickness of 40Å. This passive layer is responsible for the corrosion resistance and the hydrophobicity of stainless steel.

In this context, in the case of initial adhesion, the more hydrophobic the bacterial cell, the greater its ability to bind directly to this surface. Similar considerations were observed by Meylheuc et al. (2006) and Sheng et al. (2007).

Thus, surfaces considered hydrophobic, such as stainless steel, allow the adhesion to occur more easily than less hydrophobic or hydrophilic surfaces, which is evidenced by counts and electron micrographs, which show improved adhesion of the cells on the surface of stainless steel at the end of 240 h incubation compared to the polypropylene surface.

In studies conducted by Boari et al. (2009), that consisted of evaluating *S. aureus* biofilm formation on stainless steel using milk as substrate and different growing conditions, biofilm formation by *S. aureus* was observed by scanning electron micrographs in all conditions tested, revealing the adhesion ability of this bacterium mainly to the stainless steel surface, which was also observed in electron micrographs of the present study.

In a review by Chmielewski and Frank (2003) it is shown that a layer of organic matter on the surface can promote and facilitate bacterial adhesion. Moreover, these authors state that the time of contact between cells and surfaces also influence the bacterial adhesion. The

**Table 3.** Number of sessile cells (Log CFU.cm<sup>-2</sup>) of *Staphylococcus aureus*, quantified during biofilm formation on of AISI 304 stainless steel and polypropylene surfaces.

Time (h)	Stainless steel (Log CFU.cm <sup>-2</sup> )	Polypropylene (Log CFU.cm <sup>-2</sup> )
48	6.13 ± 0.42 <sup>a</sup>	6.13 ± 0.54 <sup>a</sup>
96	6.17 ± 0.33 <sup>a</sup>	6.12 ± 0.48 <sup>a</sup>
144	6.57 ± 0.44 <sup>a</sup>	6.56 ± 0.49 <sup>a</sup>
192	6.89 ± 0.17 <sup>a</sup>	6.64 ± 0.49 <sup>a</sup>
240	7.38 ± 0.12 <sup>b</sup>	6.58 ± 0.05 <sup>a</sup>

\*\*Results are expressed as mean ± standard deviation. Means followed by different letters in the same line differ by the Tukey test at 5% probability.

irreversible cell adhesion to surfaces occurs between 20 min and a maximum of 4 h of contact. After this period the removal of these cells requires the application of physical force, chemicals or heat. In this present study it is possible to observe that the bacterial cells have obtained adhesion to the stainless steel and polypropylene surfaces from 48 hand increasing, to a small extent, up to 240 h.

The probability of cells remaining irreversibly attached after sanitation procedures is high and corresponds to one of the main reasons for the formation of biofilms on surfaces that come into contact with food, becoming a constant source of contamination.

Table 3 presents the counts of sessile cells adhered to the surfaces of the AISI 304 stainless steel and polypropylene coupons after treatment with the control sanitizing solution and the essential oil-based sanitizing solutions. Table 4 shows the reduction percentage of the number of sessile cells after treatment with the sanitizing solutions.

The effectiveness of the sanitizing solutions containing essential oils can be observed by the counts obtained after treatment of coupons on both surfaces and the reduction percentage of these cells. A significant difference in the counts and reduction percentage of adhered sessile cells can be noted among the different treatments (Table 5). All sanitizing solutions based on essential oils showed more superior antimicrobial activity than the control sanitizing solution.

The effectiveness of the sanitizing solutions based on *S. aromaticum*, *T. vulgaris*, and their combination differ significantly from each other, their combination being the most effective to reduce the number of sessile cells adhered to the surfaces. It can be observed that the 5 min exposure of the coupons containing the biofilm to the sanitizing solution based on the combination of oils was effective to promote non-recovery of viable cells adhered to both surfaces. Scanning electron micrographs of

Figure 2 show a reduction of *S. aureus* cells on surfaces stainless steel of AISI 304 and polypropylene.

The sanitizing solution based on *T. vulgaris* was more effective compared to *S. aromaticum*. This solution allowed the non-recovery of viable cells after exposure for 10 min to both surfaces. The sanitizing solution based on the essential oil of *S. aromaticum* was less effective, presenting a reduction in the number of sessile cells, but after 10 min of stainless steel and polypropylene coupon exposure to this solution, viable cells were still recovered.

As *S. aureus* is Gram positive, it is concluded that the cell wall does not serve as a barrier to the entrance of such antibacterial compounds through the cytoplasmic membrane. Since the cell wall of these bacteria is permeable, usually it does not restrict the penetration of these sanitizing agents (Schaffer and Messner, 2005).

The difference between the performance of the sanitizing solutions within each phase of biofilm formation analyzed can be attributed to environmental and growth factors that are related to the concentration and nature of the chemical constituents, such as composition, functional groups and the structural configuration of the essential oil components (Chang et al., 2001).

Marques et al. (2007) show in their work that *S. aureus*, when in the form of biofilm, becomes more resistant to sanitizing agents.

The effects of colonization of surfaces where food is processed can result in various problems, been of an economic or public health nature. On the economic front, spoilage bacteria can contaminate food by changing its characteristics and resulting in economic losses. The risk to public health is the most serious problem, because the biofilm can transport pathogenic microorganisms and be a source of chronic contamination (Ribeiro-Furtini, 2005).

This multidisciplinary study led to the description of the sanitizing solutions essential oils of *S. aromaticum* and *T. vulgaris* and their combination on biofilm formed by *S. aureus* (ATCC 25923) on AISI 304 stainless steel and polypropylene surfaces. All solutions showed potential antibacterial sanitizers, being effective in reducing bacterial biofilms on these surfaces. The solution containing the combination of essential oils was more efficient by reducing 7.38 and 6.58 Log CFU.cm<sup>-2</sup> cells adhered on the surfaces of AISI 304 stainless steel and polypropylene respectively, after 5 min of contact.

For the analysis of scanning electron micrographs obtained, were observed structural changes on the cell surface. Probably, these changes were caused by the action of essential oils in the cytoplasmic membrane, resulting in increased permeability and extravasation of intracellular constituents.

Studies by Oliveira et al. (2010) revealed, by means of electron micrographs of transmission electron microscopy, alterations in the cell wall of *Clostridium perfringens*,

**Table 4.** Number of *Staphylococcus aureus* sessile cells (Log CFU.cm<sup>-2</sup>) quantified on of AISI 304 stainless steel and polypropylene surfaces at 240 h of biofilm formation after treatment with the control sanitizing solution and the essential oil-based sanitizing solutions.

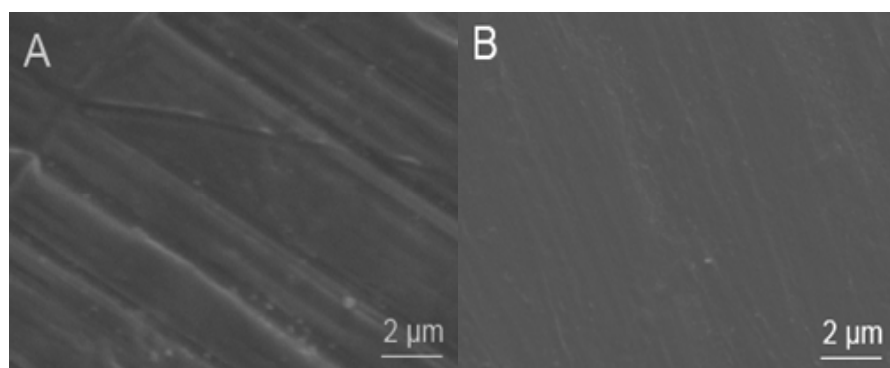
Sanitizing agent	Surfaces and exposure times			
	Stainless steel (Log CFU.cm <sup>-2</sup> )		Polypropylene (Log CFU.cm <sup>-2</sup> )	
	5 min	10 min	5 min	10 min
Control	7.09±0.06 <sup>dB</sup>	6.68± 0.27 <sup>cα</sup>	6.32± 0.0 <sup>dA</sup>	6.23± 0.03 <sup>cα</sup>
<i>S. aromaticum</i>	5.25± 0.50 <sup>CB</sup>	4.23± 0.36 <sup>bα</sup>	4.58± 0.71 <sup>cA</sup>	4.27± 0.57 <sup>bα</sup>
<i>T. vulgaris</i>	3.89± 0.62 <sup>bA</sup>	0± 0.00 <sup>aα</sup>	3.47± 0.01 <sup>bA</sup>	0± 0.00 <sup>aα</sup>
Combination	0± 0.00 <sup>aA</sup>	0± 0.00 <sup>aα</sup>	0± 0.00 <sup>aA</sup>	0± 0.00 <sup>aα</sup>

\*Results expressed as mean sessile cell counts (Log CFU.cm<sup>-2</sup>). Means followed by different lowercase latin letters in the same column differ by Tukey test at 5% probability, means followed by different uppercase latin letters on the same line differ by Tukey test at 5% probability; means followed by different Greek letters on the same line differ by Tukey test at 5% probability.

**Table 5.** Reduction of the number of *Staphylococcus aureus* cells (Log CFU.cm<sup>-2</sup>) and in percentage, quantified on the AISI 304 stainless steel and polypropylene surfaces, at 240 h of biofilm formation after treatment with the control sanitizing solution and essential oil-based sanitizing solutions.

Sanitizing agent	Surfaces and Exposure time			
	Stainless steel (Log CFU.cm <sup>-2</sup> ) (%)		Polypropylene (Log CFU.cm <sup>-2</sup> ) (%)	
	5 min	10 min	5 min	10 min
Control	0.29 - 3.9 <sup>aA</sup>	0.70 - 9.4 <sup>aα</sup>	0.26 - 3.9 <sup>aA</sup>	0.35 - 5.3 <sup>aα</sup>
<i>S. aromaticum</i>	2.13 - 28. <sup>bA</sup>	3.15 - 42.6 <sup>bβ</sup>	2.00 - 30.3 <sup>bA</sup>	2.31 - 35.1 <sup>bα</sup>
<i>T. vulgaris</i>	3.49 - 47.2 <sup>cA</sup>	7.38 - 100 <sup>cβ</sup>	3.11 - 47.2 <sup>cA</sup>	6.58 - 100 <sup>cα</sup>
Combination	7.38 - 100 <sup>dB</sup>	7.38 - 100 <sup>cβ</sup>	6.58 - 100 <sup>dA</sup>	6.58 - 100 <sup>cα</sup>

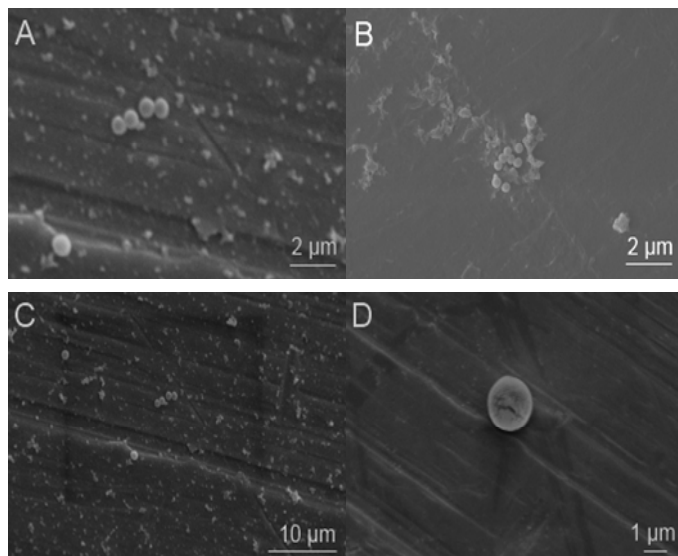
\*Values obtained from subtracting the number of adhered cells without any sanitizing treatment of stainless steel (7.38 Log CFU.cm<sup>-2</sup>) and polypropylene coupons (6.58 Log CFU.cm<sup>-2</sup>), at 240 h of biofilm formation after treatment with the control sanitizing solution and the essential oil-based sanitizing solutions. Means followed by different lowercase Latin letters in the same column differ by Tukey test at 5% probability, means followed by different uppercase Latin letters on the same line differ by the Tukey test at 5% probability; means followed by different Greek letters in the same line differ by the Tukey test at 5% probability.



**Figure 2.** Scanning electron micrographs showing a reduction of *Staphylococcus aureus* cells on the surfaces of AISI 304 (A) stainless steel and polypropylene (B).

when it was treated with essential oil of *Satureja montana*. The electron micrographs of cells not exposed to the culture that oil had continuous, smooth and thin cell

walls and cell structures were defined. Already electron micrographs of cells that were exposed to treatment with the oil showed morphological changes in the wall as



**Figure 3.** Electron micrographs scan demonstrating structural changes caused by the action of essential oils of *Syzygium aromaticum* (A and C) and *Thymus vulgaris* (B and D) in the cells of *Staphylococcus aureus* on the surfaces stainless steel of AISI 304 (A, C and D) and polypropylene (B).

roughness and irregularities, leading to rupture of the wall and subsequent cell lysis.

Furthermore, binding and clustering of intracellular material in the cytoplasm of the cells were observed because the electron micrographs revealed that these cells lacked cytoplasm in certain regions due to functional loss of the membrane caused by the action of the chemical components of *S. montana*.

Damage in cells of *C. perfringens* were also observed by Si et al. (2009), evaluated the effect of different essential oils in the morphology of bacterial cells by scanning electron microscopy, where occurred formation of cracks in the cell walls of the cultures treated with essential oils, which can also be observed in this study (Figure 3D).

The scanning electron micrographs below (Figure 3) reveal structural alterations probably caused by the action of essential oils *S. aromaticum* and *T. vulgaris* cells of *S. aureus* on surfaces stainless steel of AISI 304 and polypropylene.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

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