

Reduction of *Aeromonas hydrophila* biofilm on stainless steel surface by essential oils

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Abstract

This study demonstrates the possibility of using sanitizing detergents based on natural products for the elimination and/or reduction of *Aeromonas hydrophila* biofilm formed on stainless steel surfaces. The goal of this work was to determine the reduction effect of sanitizing detergents containing essential oils of *Thymus vulgaris* (thyme) and *Cymbopogon citratus* (lemongrass) on biofilm formed by *A. hydrophila* on AISI 304 stainless steel coupons, using UHT skimmed milk as substratum. There was adhesion and biofilm formation by *A. hydrophila* at 28 °C, presenting 7.60 log cfu.cm⁻² after the fourth day of cultivation. There was no significant difference between the lemongrass treatment and that of the thyme oil ($p < 0.05$). However, both treatments significantly reduced the biofilm, differing significantly from the NaOH control ($p > 0.05$). The treatment with lemongrass solution reduced the biofilm by 4.51 log cfu cm⁻² at 25 °C. The thyme detergent also reduced the number of cfu cm⁻² by 3.84 log cycles at 25 °C. The use of the lemongrass and thyme solutions efficiently reduced the *A. hydrophila* biofilm.

Key words: *Thymus vulgaris*, *Cymbopogon citratus*, natural disinfectants, sanitation.

Introduction

Aeromonas hydrophila is recognized for its capacity to produce several virulence factors such as cytotoxins and enterotoxins and adhesion capacity. These factors can cause hemolysis and allow the invasion of epithelial cells (Prigent-Combaret and Lejeune, 1999) causing gastroenteritis in humans. Due to its aquatic origin, it presents a ubiquitous distribution in the environment, being able to present itself in the most varied types of animal products and vegetables, such as fish and meat and its derivatives or any food that comes in contact with water (Trombetta *et al.*, 2005). *A. hydrophila* is a gram-negative, facultative aerobic, not spore former, catalase-positive, oxidase-positive and has an optimum development temperature of 28 °C (Joseph and Carnahan, 2003; Lafarge *et al.*, 2004). Due to presence of polar and lateral flagella, this bacterium presents mobility and adhesion and biofilm formation capacity (Gavin *et al.*, 2002).

Biofilms are complex microbiologic ecosystems embedded in matrixes of organic polymers adhered to a surface. Once established, sessile bacteria express genes in a model that differs greatly from that of planktonic bacteria, leading to phenotypic changes (Prigent-Combaret and Lejeune, 1999). One of those outstanding properties is the increase of sessile cell resistance to host defenses, biocides, antibiotics and various physiochemical agents (Costerton, 2005; Rota *et al.*, 2008). Therefore, biofilm cells can persist and survive even after sanitization processes, representing a possible source of food contamination and subsequent food poisoning in humans and animals (Chavant *et al.*, 2007). A large number of studies aiming to find effective elimination and biofilm control strategies have been published (Gandhi and Chikindas, 2007), demonstrating the necessity of obtaining new products with biocide action, seeking the elimination of microbial biofilms and their effective control in food industries.

The interest in natural antimicrobial compounds has been growing due to the changes of consumer attitude toward the use of synthetic food preservation, detergents and sanitizers which possess negative impacts on the environment (Danielsen *et al.*, 2008; Lebert *et al.*, 2007). Thus, the use of essential oils stands out. The action mechanisms of essential oils are little known. Considering the great number of different groups of chemical compounds present in such oils, it is observed that their antibacterial activity is not attributed to a single specific mechanism, which leads to the conclusion that they target several processes in the microbial cell (Mondello *et al.*, 2003; Ogunwande *et al.*, 2005; Suñen *et al.*, 2003).

The species *Cymbopogon citratus* (A.D.) Stapf., belonging to the family Poaceae (Gramineae), is popularly known by more than 20 names, among these lemongrass, grass-saint lemon verbena, fragrant grass, cidreira grass, lesser citronella and true cidreira herb (Cardoso *et al.*, 2000). The most expressive economical use of *C. citratus* is the production of its essential oil, rich in citral and widely used in the food, pharmaceutical and cosmetic industries (Cardoso *et al.*, 2000). Studies suggest that the antibacterial activity of *C. citratus* oil is mainly due to the α and β -citral components present in it (Oliveira *et al.*, 2010). Besides *C. citratus*, the genus *Thymus* encompasses numerous species and varieties, which have had their essential oils studied (Martins *et al.*, 2004; Onawunmi *et al.*, 1984; Sotomayor *et al.*, 2008). Its essential oil is rich in timol, presenting traces of carvacrol, scientifically recognized potent bactericides and fungicides (Essawi and Srour, 2000). More recent studies prove the antibacterial activity of *T. vulgaris* as being effective against gram-positive and Gram negative bacteria (Barbosa *et al.*, 2009).

Aimed at discovering alternate forms of eliminating biofilms formed by *A. hydrophila* on stainless steel surfaces, this work evaluated the sanitizing capacity of sodium hydroxide solutions enhanced with the essential oils of *C. citratus* and *T. vulgaris*.

Materials and Methods

Microorganism used and standardization

Aeromonas hydrophila ATCC 27853 was employed. The growth curves were prepared for standardization of the colony-forming units count. Aliquot of pure cultures (10 μ L) were transferred to Erlenmeyer flasks containing 200 μ L of BHI (Brain Heart Infusion) broth and incubated at 28 °C. Growth was monitored every hour through spectrophotometry (620 nm). The absorbance readings were taken periodically, until 10⁹ cfu mL⁻¹ were obtained.

Plant material

Dried leaves of *C. citratus* were used, obtained from the Medicinal Plant Nursery of the Federal University of Lavras, (UFPA). The collection was conducted from 8 to 9

o'clock in the morning, on sunny days, in September of 2009, under a temperature of approximately 20 °C. The *T. vulgaris* dry leaves were acquired from the local commerce of Lavras, MG, Brazil.

Extraction of essential oils and quantification of main constituents

The essential oil extraction process was conducted using the hydrodistillation method, in a modified Clevenger-type apparatus, adapted with a 4 liter round bottom balloon flask with a ground-glass mouth (Castro *et al.*, 2006).

Quantitative analysis of the constituents of the essential oils

A Shimadzu model CG-17A gas chromatograph equipped with a flame ionization detector (FID) and a fused-silica capillary column containing a bound DB5 stationary phase (30 m x 0.25 mm) were employed for quantitative analysis. The carrier gas was nitrogen, with a flow rate of 2.2 mL min⁻¹; the split ratio was 1:10, and the injected volume was 1 μ L. The temperatures of the injector and the detector were 220 and 240 °C, respectively. Three injections were performed for each essential oil tested. The apparent average concentration and standard deviation for each constituent were calculated after area normalization (Adams, 2007).

Determination of Minimum Inhibitory Concentration (MIC) of essential oils

The antimicrobial activity of the essential oils was tested through the agar plate well diffusion method proposed by Deans and Ritchie (1987) and modified by Pereira *et al.* (2008). The inoculum was standardized at the final concentration of 10⁸ cfu mL⁻¹. Solutions of *C. citratus* and *T. vulgaris* oils were used in absolute ethanol at the concentrations of 0 (control), 4, 8, 16, 31, 62, 125, 250 and 500 μ L mL⁻¹. The plates containing the inoculum and the different oil concentrations were incubated at 28 °C for 24 h (Onawunmi, 1984). The diameters of the inhibition zones were measured in millimeters. Ethanol without addition of essential oil was used as control as used in related studies to antibacterial activity using ethanol as an oils diluent (Chorianopoulos *et al.*, 2008; Oliveira *et al.*, 2010; Rota *et al.*, 2008). All tests were performed in triplicate with three replications.

Sanitization of coupons

In order to initiate the bacterial cell adhesion stage, the coupons and stainless steel passed for a previous process of hygiene and sterilization. First they were cleaned with acetone 100%, rinsed with sterilized distilled water, dried and cleaned with alcohol 70% (v/v). After that, they were washed with sterilized distilled water, dried for 2 h at 70 °C and autoclaved at 121 °C for 15 min.

Adhesion of bacterial cells on stainless steel surface

The experimental model of biofilm formation by *A. hydrophyla* was elaborated based on a system first used by Marques *et al.* (2007). In the present study, the experimental model consisted of 20 stainless steel coupons AISI 304 (1x10x20 mm) and in Petri dishes 140 mm diameter containing approximately 25 mL of UHT skimmed milk. AISI 304 was chosen because it is one of the most used stainless steel. The plates were incubated at 28 °C for 48 h and agitated at 50 rpm.

Enumeration of adhered bacterial cells

Initially, 60 mL of TSB previously sterilized and aliquot of TSB containing the bacterial culture were added to the Petri dish at a final concentration of approximately 5 Log cfu mL⁻¹. The system containing the coupons was sealed and incubated at 28 °C under 50 rpm agitation. Every 48 h, the coupons were removed from the base and immersed three times into a saline solution to remove the planktonic cells, and again placed in a new sterilized Petri dish, which was immersed in 60 mL of TSB. This procedure was repeated every 48 h, completing 240 h of incubation, to form a mature biofilm.

Every 48 h the bacterial cells adhered to the coupons were removed using the smear technique. We used standardized sterile swabs (100 times). The swabs were immersed in tubes containing 10 mL of peptone water 0.1% (w/v) and subjected to intense agitation Vortex for 3 min. Serial dilutions in peptone water 0.1% (w/v) were made from the test tube containing the swab, followed by plating aliquots of 100 µL on the surface of TSA (Silva *et al.*, 2010). The plates were incubated at 28 °C for 24 h. Analyses were performed every 48 h. The entire procedure was performed in triplicate with three replicates.

Aeromonas hydrophyla biofilms treatment with essential oils and alkaline detergent

After 240 h, was performed the treatment of biofilms with the disinfectant solutions-based essential oils. The coupons were previously immersed three times in the saline solution to remove the planktonic cells. After were immersed in solutions: NaOH solution (1% w/v) with addition of sterile water in place of essential oil, sterilized distilled water containing NaOH solution with essential oil of *C. citratus* and NaOH solution containing essential oil of *T. vulgaris*, both oils diluted in absolute ethanol at MIC concentrations determined before, 31 µL mL⁻¹ and 62 µL mL⁻¹, respectively. The solutions containing the coupons were kept for 15 min (contact time was chosen considering the usefulness of these solution in the process of hygiene at food industries) temperature 20 °C (Andrade and Macêdo, 1996). After this period the coupons were removed from the solutions, rinses with salt water 3 times and subjected to smear with the swabs and dilution procedure and plating as already described in previous item.

Scanning Electron Microscopy Analysis (SEM)

The preparation of the coupons for observation via scanning electron microscopy followed the protocol proposed by Alves (2004). All of the procedures were carried out at the Electronic Microscopy and Ultra structural Analysis Laboratory of the Federal University of Lavras, (UFLA), Lavras, Minas Gerais, Brazil.

Statistical analysis

For the surviving bacteria, the experiment was conducted according to a completely randomized design, with three repetitions. In the treatments were made up of three types of detergent-sanitizers (NaOH, solution of lemongrass essential oil and NaOH, and thyme essential oil added to NaOH) and one temperature (20 °C). Counting of colony forming units cfu cm⁻² were carried out, before and after coupon immersion in the solutions. The results were expressed in the logarithmic form (log cfu cm⁻²), the variable being the measure of the ratio (proportion) between the pre-immersion log cfu cm⁻² and post-immersion log cfu cm⁻². The analysis was made using the R Development Colors Team (2008) statistical package. The factors, when significant, were appraised by the Scott-Knott test.

Results

Main components of essential oils and Minimum Inhibitory Concentration

Under the conditions used in this study for extraction of essential oils from lemongrass, the main components of the lemongrass were: neral (32.73%), geranial (46.03%) and myrcene (8.88%) (Table 1). The thyme oil was composed mainly by: 1,8 cineole (53.46%) and canfor (7.15%) (Table 2).

In this study it was found that antimicrobial activity of the essential oils were drawn on the bacteria, the MIC of essential oil of lemongrass, 31 µL mL⁻¹ was lower than that of thyme, 62 µL mL⁻¹ (Figure 1).

Table 1 - Chemical composition of essential oils of *Cymbopogon citratus*.

Constituintes	Tr ^a	% ^b
Myrcene	12.474	8.88
Linalool	16.601	2.23
Verbenol	18.998	1.62
Neral	21.909	32.73
Nerol	22.318	4.07
Geranial	22.989	46.03
Nonyl methyl ketone	23.597	1.56
Methyl undecyl ketone	30.133	1.16
Other		1.72
Total	-	100.00

^aRetention time. ^bThe percentages concern the integration of the areas of the peaks.

Adhesion of bacterial cells on the stainless steel surface

Aeromonas hydrophila biofilm was grown for 10 days for the training of the mature biofilm (Boari et al., 2009). Selection of *A. hydrophila* was because today is considered an emerging microorganism in food security and stability. This species are psychotropic microorganisms, or even presenting as a growth optimum temperature somewhere around 28 °C, are able to grow at the temperatures used in food preservation (Boari et al., 2009).

Aeromonas hydrophila adhered to the AISI 304 stainless steel surface using the UHT skimmed milk as substrate

Table 2 - Chemical composition of essential oil of *Thymus vulgaris*.

Constituents	Tr ^a	% ^b
α-tujona	10.257	0.62
α-pineno	10.795	4.17
Camphene	11.765	4.20
Sabinene	11.856	4.36
β-Pinene	12.449	2.51
Myrcene	13.711	1.56
ρ-Cimene	13.872	3.79
Limonene	14.102	3.30
1 8-Cineole	18.288	53.46
Canflor	19.064	7.15
Isoborneol	19.519	2.30
Terpinen-4-ol	19.980	5.46
α-terpineole	23.349	1.72
Bornyl acetate	26.424	2.38
Copaene	26.424	2.38
Other		0.64
Total	-	100.00

^aRetention time. ^bThe percentages concern the integration of the areas of the peaks.

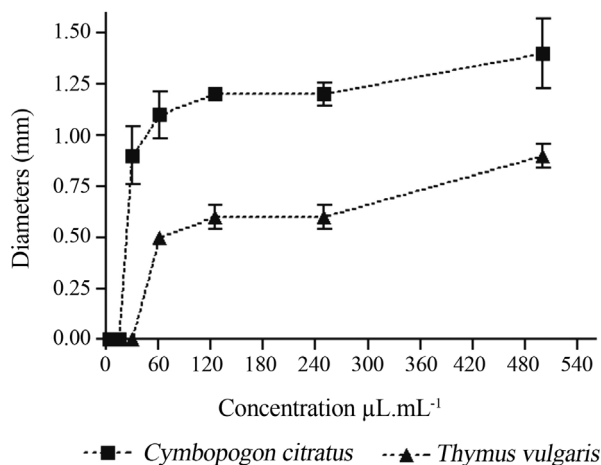


Figure 1 - Inhibition zones by different concentrations of essential oils of *Cymbopogon citratus* and *Thymus vulgaris* on *Aeromonas hydrophila*.

tum and formed the biofilm. After two days of cultivation, the bacterium had already formed biofilm on the surface, reaching 6.6 log cycles cfu cm⁻², and after 10 days of cultivation the number of sessile cells increased to 7.8 log cfu cm⁻². Such increase occurred due to the multiplication of the adhered cells, since the milk was replaced every two days with a new sterilized base.

Although the cultivation lasted 10 days, the number of cfu cm⁻² increased slightly more than 1 log cycle, showing that, when cultivated at 28 °C and in skimmed milk, *A. hydrophila* did not form thick biofilm.

Disinfectant and biocide action against *Aeromonas hydrophila* biofilm

For the evaluation of the detergent-sanitizing solutions' efficiency (essential oil and NaOH 1%), the MIC of the thyme essential oil (62 μL mL⁻¹) and the lemongrass oil's (31 μL mL⁻¹) were used. The results were expressed by the number of log cycles of cfu cm⁻² of the bacterial population remaining on the coupons after the treatments. The efficiency of the detergent-sanitizing solutions of thyme essential oil, lemongrass essential oil and that of the alkaline detergent solution (control, NaOH 1%), were compared as to the sessile cells removal capacity of the mature biofilm. The treatments were carried out at temperature 25 °C.

The detergent-sanitizing solutions of thyme essential oil, lemongrass essential oil and the alkaline detergent solution reduced bacterial population, resulting in lower log cfu cm⁻² values, in comparison to the mature biofilm without treatment (Figure 2).

There was significant difference between the treatments with alkaline detergent (56.05%) and with the sanitizing-detergent (p < 0.05). However, the difference between the percentages of cfu cm⁻² obtained through the treatment with thyme's sanitizing-detergent (42.33%) and those observed on the lemongrass (34.67%) was not significant (p > 0.05). The microbial population of the biofilm

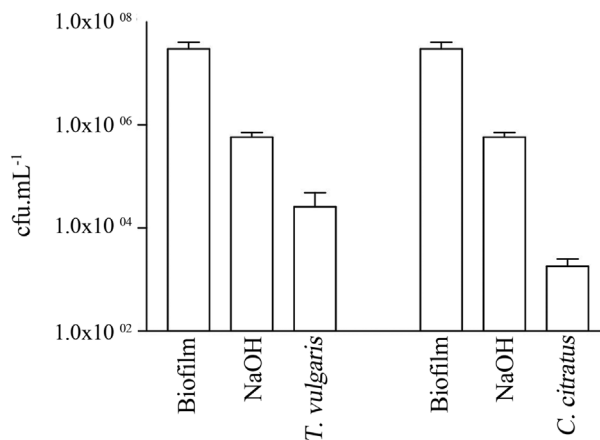


Figure 2 - Concentration of *Aeromonas hydrophila* (log CFU cm⁻²) quantified on steel coupons with and without treatments with essential oils of *Cymbopogon citratus* and *Thymus vulgaris*.

without treatment was 7.63 log cfu cm⁻². Comparing the cfu cm⁻² concentrations obtained on the coupons treated with detergent-sanitizer solution plus the lemongrass essential oil at 25 °C with those on the mature biofilm, it was observed that there were reductions of 4.51 log cfu cm⁻² on the population of *A. hydrophyla*. The thyme essential oil detergent-sanitizer also promoted reductions on population of 3.84 at 25 °C. In biofilms treated only with the solution containing NaOH was smaller reduction: 2.38 log cycles. In the Figure 3A and 3B can be observed that the biofilm was cohesive and uniform and there was the production of exopolysaccharides (EPS) and demonstrated the wide grip and different layers. In the micrographs one can observe that after the different treatments, there was breakdown of the EPS and dispersion of cells (Figure 3C and 3D).

No significant interaction between treatments of biofilms between the detergent-sanitizing solution of lemon grass and thyme (Table 3). The concentration 1% (p/v) NaOH alkaline solution reduced the biofilm cell concentration. However, the highest percentage of surviving bacteria (56.05%) was obtained. The solutions containing essential oils were more effective in reducing the viable cells in biofilms (Table 3).

Discussion

Several studies have reported the existence of different chemical characteristics between the essential oils of different species and even between identical species, but grown in different geographical regions of the globe. The main components found in the *C. citratus* oil, neral and geranial, are monoterpenes, whose antibacterial action is proven (Millezi *et al.*, 2012a; Millezi *et al.*, 2012b; Ogunwande *et al.*, 2005). However, myrcene is an acyclic hydrocarbon, not possessing antibacterial action that polymerizes and resinifies when exposed to light (Onawunmi *et al.*,

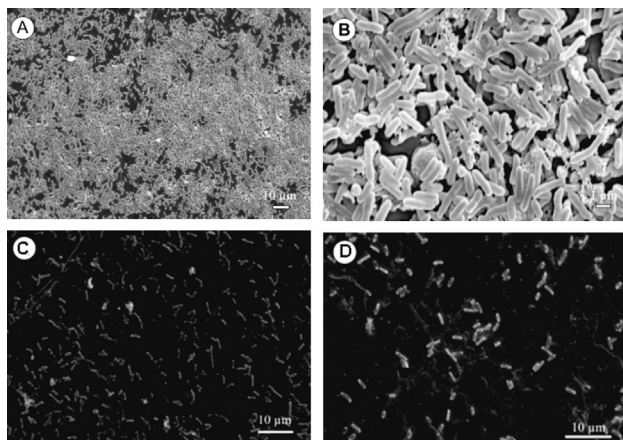


Figure 3 - Scanning Electron Micrographs: (A e B) Biofilm of *Aeromonas hydrophyla* on stainless steel coupons after a period of 10 days and EPS formation (observe the circles); (C) Treatment of the biofilm using the thyme detergent-sanitizer solution and (D) Treatment of the biofilm using the lemongrass detergent-sanitizer solution.

Table 3 - Percentage of CFU cm⁻² of *Aeromonas hydrophyla*, observed after treatment of stainless steel coupons containing biofilm, with sanitizer-detergent and detergent solutions.

Detergent* and Sanitizer-detergents	CFU cm ⁻² recovered (%)	
NaOH (1%)*	56.05	A
NaOH (1%) and thyme	42.33	B
NaOH (1%) and lemongrass	34.67	B

Averages followed by the same letter do not differ among themselves, by the Scott-Knott test, at 5% significance.

1984). These results are similar to those reported by Oliveira *et al.* (2010), for *C. citratus* essential oil, the major constituents found were: geranial (42.91%) and neral (30.90%). The 1,8 cineol, a monoterpenoid compound, largely present in the thyme oil used in this work, also possesses antibacterial activity (Gilles *et al.*, 2010), and has already been related as a possible main constituent of the thyme essential oil, depending on the stage of the plant development (Jordán *et al.*, 2006). Differences in the composition of essential oils from plants of the same species are related to several factors that may be primarily genetic and environmental, climate, altitude, soil type, rainfall and others (Gobbo-Neto and Lopes, 2007).

The fact that *C. citratus* essential oil presented a high concentration of terpenoids suggests that such substances contributed to the high biocide performance of that essential oil at a lower concentration than that of the *T. vulgaris* essential oil. The antibacterial activities on *Staphylococcus aureus* and *Escherichia coli* were shown by Pereira *et al.* (2008). Works with *T. vulgaris* showed antibacterial activity on food spoiling and pathogenic bacteria, Gram negatives, such as *Salmonella* Typhimurium, there was formation of inhibition zones of 30 mm, there was high sensitivity to essential oil (Rota *et al.*, 2008). In our study with the Gram negative *A. hydrophyla*, the results obtained are consistent with those found in literature.

The antibacterial action of the terpenes (main components of the lemongrass and thyme) is not completely understood. It is known, however, that it involves the rupture of the bacterial cytoplasmic membrane due to the lipophilic nature of such substances. The aforementioned interaction causes a fluidity and permeability increase of the cell membrane, the destruction of the membrane's proteins, inhibition of cell respiration and ion transport alterations (Cowan, 1999; Burt and Reinders, 2004; Woodling and Moraru, 2005).

Although the antimicrobial activity of the essential oils of various plants has been related, these works used planktonic cells, and few studies can be found in which the capacity of such oils to reduce biofilm on stainless steel surfaces were evaluated. Another importance factor of this study was the combined use of alkaline detergents and essential oil solutions, seeing as similar works cannot be found on literature.

Aeromonas hydrophila is microorganism common in raw milk in cooling tanks at 4 °C, with an increase in numbers after 24 h of storage of the milk (Lai *et al.*, 2007). Once present, it can readily adhere to the stainless steel of the tank, producing biofilm (Lynch and Roberteson, 2002). In fact, the speed with which the cells adhere to the surface, forming biofilm which occupies the stainless steel's surface in a homogeneous way as was shown. Although the temperature used for the biofilm formation has been the optimum growth temperature for *A. hydrophila* that behavior still occurs even under inadequate conditions for its multiplication. Boari *et al.* (2009) showed that *A. hydrophila* formed biofilm when cultivated in UHT skimmed milk under 4, 7 and 18 °C, and that at 7 and 18 °C the biofilm had already been formed on the second day of cultivation, with counting of 5,14 log cfu cm⁻² and 6,67 log cfu cm⁻², respectively. Steinberger and Holden (2005) showed that, the richer the environment where the *Pseudomonas aeruginosa* develops, the faster the biofilm forms. As the milk used was skimmed in that work, one may infer that its nutritional characteristics influenced in the rapid formation of the biofilm.

The milk, during the experiment, was replaced every 48 h, providing fresh substratum, in order to avoid inhibition of the microorganism metabolic activity due to limitation of growth factors. The depletion of those factors occurs; therefore the consumption of the substratum or final electron acceptors occurs at a rate superior to that of biofilm replacement (Burt and Reinders, 2004). That happens because of the lower compound diffusion in the exopolysaccharides matrix, making them less available to the bacteria.

In spite of the sessile cells presenting differentiated microbial agent resistance characteristics (Werner *et al.*, 2004), the antimicrobial potential of those substances on the cells is determined using planktonic cells, being one of the recommended techniques to determine the minimum inhibitory concentration in diffusion in agar. Thus, that methodology was employed with the biocide agents proposed in this work obtaining the inhibition zones and the oil concentrations that were used in the elaboration of the detergent-sanitizing solutions for evaluation of their activity on the *A. hydrophila* biofilm.

The detergent solution (alkaline solution) removed part of the *A. hydrophila* biofilm. However, its effectiveness was significantly lower compared to the treatments conducted with the sanitizing detergent solutions. In the Figures 3C and 3D can be seen that the essential oils breakage caused in the structure of EPS and dispersion of bacterial cells. Chorianopoulos *et al.* (2008) tested the activity of acid and base chemical disinfectants and also that of the essential oil of *Satureja thymbra* on biofilms formed by bacterial species on stainless steel coupons. The results showed that the *S. thymbra* essential oil presented satisfactory antimicrobial action on the biofilm formed by *Salmonella enterica*, with reduction of 4 log cycles cfu cm⁻². In the

present research, similar results were observed. The treatment with the thyme and lemongrass detergent-sanitizing solutions reduced *A. hydrophila* by 4 log cfu cm⁻². In spite of not having used the combination of the essential oils, Oliveira *et al.* (2010) showed that a solution containing essential oils of *C. citratus* and *C. nardus* can reduce *Listeria monocytogenes* adhered cells on stainless steel by 100%.

The sanitizers used in industry and in laboratory tests under conditions indicated by manufacturer, are approved in tests as suspension and use dilution, when they promote 5 decimal reductions (DR) after 30 seconds of contact at 20 °C. But, in culture media, solid or liquid, there is no glycocalyx formation, fundamental to the adhesion process and protection of the cells on biofilm (Andrade and Macêdo, 1996). However, there is no established DR standard to consider a sanitizer as effective when the tested cells are sessile. Thus, the study chose to use time of 15 min of contact with the biofilm base sanitizing solutions of essential oils.

Some works such as the one of Lebert *et al.* (2007) investigated the efficiency of thymol and eugenol solutions and quaternary ammonium salt based sanitizer, and *S. thymbra* essential oil on multispecies biofilm formed by *Pseudomonas fragi* and *Escherichia coli* and pathogenic biofilm formed by *S. aureus* and *L. monocytogenes*. The quaternary ammonium salt is a traditional bactericide used in food industry. When the essential oil of *S. thymbra* was used at 1% and 2%, there was reduction of the spoilage bacteria of 5.1 log for *E. coli* and 5.2 for *L. monocytogenes*, but the pathogenic bacteria were not reduced sufficiently: 1.3 log for *S. aureus* and 2.5 log for *L. monocytogenes*. With the combination of the quaternary ammonium compound with thymol and/or eugenol, a reduction in the inhibition of the biofilm was observed. According to the authors, the thymol did not present solubility in the compound based on the quaternary ammonium salt forming an emulsion to promote the mixture of the compounds.

According to the results demonstrated in this research, in spite of there not being significant statistical difference between the thyme and lemongrass sanitizer-detergents, the lemongrass sanitizer-detergent promoted a reduction of 1 log above that obtained with the thyme, which was considered relevant. Keeping in mind the conditions of the medium, the microorganism in question and its generation time, this difference is important, because it can result in more efficient mitigation of the contamination of products and of the transmission of diseases. Thus, we suggest further research on search strategies using natural antimicrobials against bacterial biofilms, there are few studies on this perspective, but the studies already conducted have shown promising results.

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