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APPROACHES TO REMOVAL AND KILLING OF *SALMONELLA* SPP. BIOFILMS

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ABSTRACT

This study aims to assess the effectiveness of three sanitizers (an alkaline solution, an acid solution and a quaternary ammonium salt) to remove biofilm formed by *Salmonella* spp. on stainless steel surfaces. To evaluate the individual or combined effects of sanitizer concentration, time (5.0–25 min) and temperature (25–65C) on survival of *Salmonella* spp. biofilms three different Central Composite Designs (CCD) were developed. Results highlighted that treatments containing NaOH were able to eliminate more than 82–83% of sessile population, whereas the total removal of biofilm was obtained using a solution at least 800 ppm for 15 min at 45C. Peracetic acid seemed to be more effective than NaOH assuring the total removal of biofilm at low temperature. Even the quaternary ammonium salt was able to remove completely *Salmonella* spp. biofilms at 60C using a solution at 12 ppm, or at higher temperature (65C), using a less concentrated solution (6 ppm).

PRACTICAL APPLICATIONS

Due to the wide range of contributing factors (surface type, availability of nutrients and oxygen, microbial species, etc.) biofilms are quite diverse; in essence it can be said that each biofilm is different. Even if numerous attempts have been made to find standardized systems to prevent, remove and kill biofilm cells, until now there is no unique system that is able to remove all biofilms. Thus, a study on the factors affecting the resistance of *Salmonella* spp. biofilms against sanitizers may be useful into the development of new sanitation strategies in food industries.

INTRODUCTION

The term "biofilm" is referred to a functional consortium of micro-organisms attached to a surface and embedded in extracellular polymeric substances (EPS) produced by the same micro-organisms; the definition includes microbial aggregates, flocculates and population adherent to membrane pores (Somers and Wong 2004; Pan *et al.* 2006; Chaitiemwong *et al.* 2010).

Biofilm formation is a very complex process and consists of an initial attachment, micro-colonization and EPS production, followed by maturation. In the first step several factors, such as substratum composition, surface chemistry and topography, fluid flow and low nutrient availability, are able to induce phenotypic changes of planktonic (free living) cells to the sessile form (attached cells) in a very short time (Chmielewsky and Frank 2003; Shi and Zhu 2009). The EPS, containing polysaccharides, proteins, phospholipids, teichoic and nucleic acids, and other polymeric substances hydrated from 85 to 95% water, are very important because protect the biofilm inhabitants by concentrating nutrients, preventing access of biocides, sequestering metals and toxins and preventing desiccation (Somers and Wong 2004; Pan *et al.* 2006; Chaitiemwong *et al.* 2010). Mature biofilm may consists of a single layer of cells in porous extracellular polymer or multilayered loosely packed microcolonies held together with EPS and interspersed with water channels (Chmielewsky and Frank 2003), fundamental for the passage of the exchange of nutrients, metabolites and waste products (Corbo *et al.* 2009).

A biofilm can be formed everywhere (toilets, sinks, industrial water systems and on medical devices) by several types

of microorganisms, including spoilage and pathogenic microorganisms, such as *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica*, *Campylobacter jejuni* and *Escherichia coli* O157:H7 (Corbo *et al.* 2009). For food industry *Salmonella* spp. has considerable significance, since it is one of the most important food-borne pathogens, having the ability to attach and form biofilm on surfaces found in food processing plants, including plastic, cement and stainless steel (Chmielewsky and Frank 2003). Biofilm formation could lead to serious negative implications in human life, above all if biofilms are formed by pathogens: one of these is the increasing of the risk for microbial contamination in food plants, causing post-processing contamination and leading to lowered shelf-life of the food product and/or transmission of diseases (Meyer 2003; Corbo *et al.* 2009).

From this point of view, cleaning and sanitizing treatments acquire great importance, in order to prevent or completely destroy the biofilm. Cleaning procedures should effectively remove food debris and other soils that may contain microorganisms or promote microbial growth, through cold or warm water followed by the application of chemical agents, rinsing and sanitation (Frank 2000).

The sanitizers generally used in the food industries are peroxygens, alkalines, acids and quaternary ammonium compounds, which have to penetrate the matrix of EPS and gain access to the microbial cells causing biofilm inactivation and removal (Simoes *et al.* 2010).

Unfortunately it is very difficult to eliminate the biofilms, because some their properties, such as reduced diffusion, physiological changes, enzyme mediated resistance and genetic adaptation (Cloete 2003; Corbo *et al.* 2009), confer resistance to antimicrobial treatments and unfavorable environmental factors (Simoes *et al.* 2010).

One strategy to prevent the formation of biofilm is to disinfect regularly, before biofilm formation starts. However, the initial phase of biofilm formation, i.e., the attachment of microbes to a surface, is rather a fast process and takes only a few hours. It is mainly in this initial phase when phenotypic adaptation is developed (Das *et al.* 1998). Moreover, in many application fields, e.g., in the food industry, it is hardly possible to disinfect frequently enough to avoid this initial step.

Due to the wide range of contributing factors (surface type, availability of nutrients and oxygen, microbial species, etc.) biofilms are quite diverse; in essence it can be said that each biofilm is different. Numerous attempts have been made to find standardized systems to prevent, to remove and to kill biofilm cells (Joseph *et al.* 2001; Peng *et al.* 2002; Ammor *et al.* 2004). Nevertheless, until now there is no unique system which is able to remove all biofilms.

This study aims to assess the effectiveness of three sanitizers (an alkaline solution, an acid solution, a quaternary ammonium salt) to remove biofilm formed by *Salmonella* spp. on stainless steel surfaces. In order to evaluate the individual or combined effects of sanitizer concentration, time (5.0–25 min) and temperature (25–65C) of exposure on survival of *Salmonella* spp. biofilms, three different Central Composite Designs (CCD) were developed.

MATERIALS AND METHODS

Biofilm Formation

A strain of *Salmonella Enterica* (ATCC 35664) was used as test organism. The strain was stocked at −20C in Tryptone Soya Broth (TSB, Oxoid, Milan, Italy) with the addition of 33% of glycerol. Prior to use, a working culture was activated by two successive 24-h transfers of cell in TSB broth at 37C. The surface used to get the biofilm attached was stainless steel (AISI-316, finish#2B, ARVEL, Naples, Italy) and was cut into 2.5 × 5.0 × 0.05 cm rectangular chips. This material has been reported as the ideal one for food processing, since it is chemically and physiologically stable at various food-processing temperatures, easy to clean and has a high resistance to corrosion (Verran *et al.* 2001).

Before each experiment, coupons were prepared by rinsing in acetone for a minimum of 30 min, rinsing in distilled water and then soaking in 1 N NaOH for 1 h. After a final rinse in distilled water, the chips were allowed to air dry. This cleansing was required to remove fingerprints, oils grease and other soils that may have been on the stainless steel (Speranza *et al.* 2011).

To allow biofilm formation, Coplin jars containing 20 mL of sterile Bacteriological Peptone (0.5 g/L, pH 6.5; Oxoid) and five sterile stainless steel chips were inoculated with the test culture (initial inoculum $\sim 10^3$ CFU/mL). The chips were placed vertically to allow the biofilm growth on both sides. All flasks were incubated at 30C, without agitation, for 7 days. The biofilm formation was encouraged following the method suggested by Speranza *et al.* (2011) who found that a greater adhesion of *Salmonella* spp. was obtained when this strain was left to grow in poor media (1.0–1.5 g/L of peptone), with an incubation temperature of about 30C, and pH close to 6.0.

Treatments and Cell Enumeration

In order to evaluate the efficacy of three different sanitizers against biofilm *Salmonella* spp., three 5 levels–3 variables CCD were developed (Bevilacqua *et al.* 2010). The three variables were sanitizer concentration, time and temperature of exposure. The five levels of each variable are reported in Table 1. NaOH (solution 2%), peracetic acid (solution 40%; Sigma-Aldrich, Milan, Italy) (PA) and Dequalinium chloride, a quaternary ammonium salt (QAC) (Sigma-Aldrich),

TABLE 1. LEVELS OF THE INDEPENDENT VARIABLES OF THE CCD CONCERNING THE TREATMENTS WITH NAOH, PA AND QAC

Levels	[NaOH] (ppm)	(pH)	Time of exposure (min)	Temperature of exposure (C)
−2	5	8	5	25
−1	10	9	10	35
0	15	10	15	45
+1	30	11	20	55
+2	120	12	25	65

Levels	[PA] (ppm)	(pH)	Time of exposure (min)	Temperature of exposure (C)
−2	5	6.0	5	25
−1	40	5.5	10	35
0	200	5.0	15	45
+1	250	4.5	20	55
+2	800	4.0	25	65

Levels	[QAC] (ppm)	(pH)	Time of exposure (min)	Temperature of exposure (C)
−2	4.0	6.5	5	25
−1	4.5	6.5	10	35
0	5.0	6.5	15	45
+1	5.5	6.5	20	55
+2	6.0	6.5	25	65

were used as sanitizers. The different treatment solutions were prepared in distilled water and filtered (0.2 am filter; Minisart mod.16534-K, Sartorius, Goettingen, Germany).

After 7 days of adhesion, the chips with biofilm were aseptically removed from the medium and exposed in the appropriate treatment solution (or sterile medium culture

TABLE 2. COMBINATIONS OBTAINED IN THE THREE DEVELOPED CCD

Combination	[NaOH]	[PA]	[QAC]	Time of exposure (min)	Temperature of exposure (C)
1	9	4.5	3	10	35
2	9	4.5	3	20	35
3	9	4.5	3	10	55
4	9	4.5	3	20	55
5	11	5.5	9	10	35
6	11	5.5	9	20	35
7	11	5.5	9	10	55
8	11	5.5	9	20	55
9	10	5.0	6	15	45
10	10	5.0	6	5	45
11	10	5.0	6	25	45
12	10	5.0	6	15	25
13	10	5.0	6	15	65
14	8	4.0	0	15	45
15	12	6.0	12	15	45
16	10	5.0	6	15	45
17	10	5.0	6	15	45

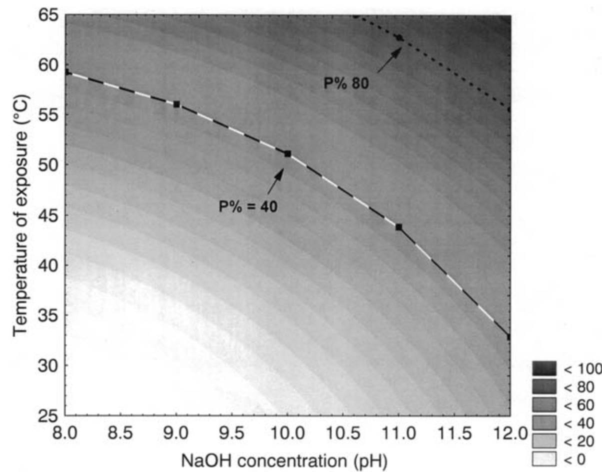

FIG. 1. "CONTOUR PLOT" OF THE INTERACTION OF NAOH CONCENTRATION × TEMPERATURE OF EXPOSURE ON THE PERCENTAGE OF CELLS MORTALITY (*P*%)

for control), one chip per test tube, without stirring. Time and temperature of exposure varied according to the three CCD (Table 2). After each treatment, the slides were aseptically removed and rinsed with sterile distilled water to remove unattached cells. Surviving sessile cells were detached from slides in a sterile test tube containing 50 mL of sterile saline with a 20Hz "Vibra Cell" sonicator (SONICS, Newcastle, CT) for 3 min. Viable and culturable cells were enumerated by serial dilution in 0.9% NaCl solution and plating on Tryptone Soya Agar, incubated at 37C for 24 h. Results were expressed as Log CFU/cm^2.

Statistical Analysis

Each experiment was performed twice at least. The results were expressed as percentage of cell mortality (*P%*):

$$P\% = 100 - (\log N_t / \log N_0) * 100$$

where N_0 are CFU/cm^2 in the control sample and N_t are the CFU/cm^2 in the treated sample. *P*% was equal to zero when no sessile cell was removed and equal to 100% when the biofilm removal was total.

The percentage of cell mortality data were submitted to statistical analysis by a stepwise regression with the backward selection procedure (Statsoft Inc., Tulsa). The goodness-of-fit of the models obtained was evaluated by regression coefficients (*R*) and Fisher's *F* test. The effects of the independent variables on *P*% were better evaluated using bi-dimensional *contour plots* obtained by imposing a constant value (i.e., the central point of CCD) to one variable at time.

RESULTS

After 7 days of adhesion, populations of adherent *Salmonella* spp. cells on stainless steel were about 7.5–7.8 log CFU/cm^2. After each sanitizer treatment, the calculated percentages of cell mortality (*P*%) were used as input values to obtain three best fit equations describing the main, quadratic and interactive effects of independent variables (sanitizer concentration, time [*t*] and temperature [*T*] of exposure) on *Salmonella* spp. biofilm removal. These equations are reported in the following; the *R* values indicate the adequacy of the proposed models to the experimental data, whereas the values of Fisher test underline the high significance of polynomial equations.

As expected, *P*% was positively affected by time in its main term, temperature of exposure and concentration of NaOH, in their quadratic terms, and negatively by sanitizer concentration, as it can be inferred from the following equation:

$$P\% = -19.657[\text{NaOH}] + 1.723\ [t] + 1.587[\text{NaOH}]^2 + 0.020\ [T]^2$$
$$R = 0.90 \qquad F = 35.07 \tag{1}$$

In our experimental conditions, the positive term $[\text{NaOH}]^2$ could be assumed as the most significant. As expected, by the evaluation of Fig. 1, showing the interactive effects of NaOH concentration and temperature of exposure, the highest mortality values were obtained at the higher sanitizer concentrations. Drawed lines indicate *P%* values of 40 and 80%. In these experimental conditions, the total removal of *Salmonella* spp. biofilm from the stainless steel chips (*P%* = 100) was never obtained. The maximum

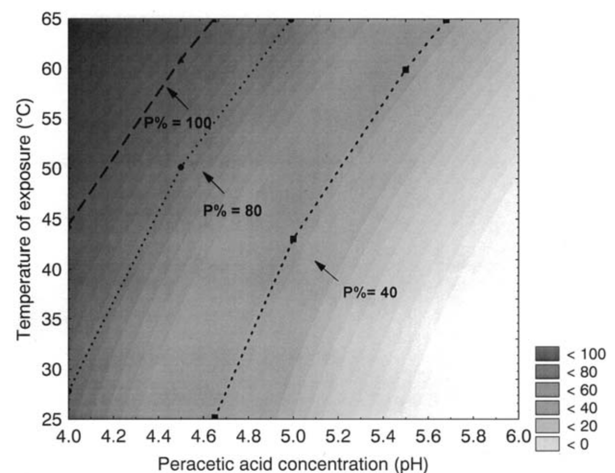

FIG. 2. "CONTOUR PLOT" OF THE INTERACTION OF PERACETIC ACID CONCENTRATION × TEMPERATURE OF EXPOSURE ON THE PERCENTAGE OF CELLS MORTALITY (*P*%)

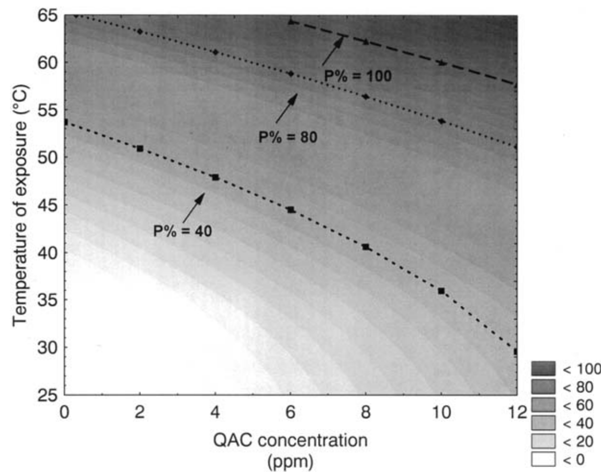

FIG. 3. "CONTOUR PLOT" OF THE INTERACTION OF QAC CONCENTRATION × TEMPERATURE OF EXPOSURE ON THE PERCENTAGE OF CELLS MORTALITY (*P*%) (C)

removal in these conditions ($P\% = 80$) was observed only at high temperatures (55–65C) with the most concentrated NaOH solutions.

About peracetic acid, *P%* was positively affected by time and temperature of exposure to this sanitizer, as main and quadratic term respectively, and negatively by the interaction [peracetic acid] × [*t*].

$$P\%=19.924[t]+0.017[T]^2-3.863[\text{peracetic acid}][t] \quad R=0.91 \quad F=51.74 \tag{2}$$

Figure 2 shows the *contour plot* relative to the effects of peracetic acid concentration and temperature of exposure. Drawed lines indicate *P%* values of 40, 80 and 100%. As expected, the highest mortality values were observed when the sanitizer solutions were at the highest concentrations. The total removal of biofilm was obtained at 45C, using a peracetic acid solution at 800 ppm (pH 4.0). The same result was also obtained at 65C, using a solution at 200 ppm (pH 4.6). Peracetic acid is a very effective antimicrobial compound; nevertheless, this molecule degrades at temperatures above 45C. Thus, in order to obtain the total removal of biofilm under 45C, it is necessary to use a solution at least 800 ppm for 15 min. In our experimental conditions, peracetic acid seemed to be more effective than NaOH to remove biofilms from stainless steel surfaces; in fact, it assured the total removal of sessile cells and it was effective at a low temperature.

Using the QAC, *P%* was positively affected by its concentration as individual term and negatively by its interaction with time of exposure:

$$P\%=12.581[\text{QAC}]-3.276[T]+0.041[T]^2-0.575[\text{QAC}][t]+0.120[T][t] \quad R=0.97 \quad F=94.61 \tag{3}$$

A better evaluation of the influence of temperature was instead achieved by Fig. 3, *contour plot* showing the interactive effects of QAC concentration and temperature of exposure. As showed in the figure, *P%* increased with the increment of temperature. As peracetic acid, even the quaternary ammonium salt was able to remove completely *Salmonella* spp. biofilms. This result was obtained at 60C using a solution at 12 ppm, or at higher temperature (65C), using a less concentrated solution (6 ppm). Compared with the other tested sanitizers, the quaternary ammonium salt used in this study (i.e., Dequalinium Chloride) seemed to be more effective than NaOH, but less active than peracetic acid. As a matter of fact, the tested concentrations were very low, so we may reasonably suppose that higher concentrations could assure the total removal of biofilm at lower concentrations and temperatures in comparison with peracetic acid.

The used approach (central composited design) was useful not only to build a second order model, on percentage of

TABLE 3. EFFECTS OF SANITIZER CONCENTRATION [*C*], TIME OF EXPOSURE [*T*] AND TEMPERATURE OF EXPOSURE [*T*] ON PERCENTAGE OF CELL MORTALITY (*P*%) OF *SALMONELLA* SPP. BIOFILM

		NaOH	*Peracetic acid*	*QAC*
Linear terms	Sanitizer concentration [*C*]	−4.486	–	3.859
	Time of exposure [*t*]	2.520	6.547	–
	Temperature of exposure [*T*]	–	–	−5.775
Interactive terms	[*C*] × [*t*]	–	−6.469	−2.732
	[*C*] × [*T*]	–	–	–
	[*t*] × [*T*]	–	–	4.092
Quadratic terms	$[C]^2$	4.314	–	–
	$[t]^2$	–	–	–
	$[T]^2$	5.288	4.095	6.784
	R^2_{ad}*	0.900	0.910	0.970

* R^2_{ad}, adjusted regression coefficient.

sessile cells mortality, but also to assign a different weight to each studied factor. In fact, the statistical weight of each term was pointed out through the standardized effects associated with each individual, quadratic and interactive factors of the equations. These effects were evaluated as the ratio of the mathematical coefficient of each term of the equation vs. the respective standard errors and are summarized in Table 3. About alkaline treatments, due to their presence as quadratic terms, temperature of exposure and sanitizer concentration were the most significant variables affecting the treatment effectiveness, whereas the effect of time of exposure was less significant. On the contrary, this variable was the most significant one, either individually and interactively with sanitizer concentration, during treatments with peracetic acid. In treatments with QAC solutions, the temperature of exposure was the most significant variable, either as individual term or in interaction with time of exposure.

DISCUSSION

Many authors have demonstrated that bacteria forming biofilm resist in a better way to different stresses, such as pH variations (Leriche *et al.* 2003) and antimicrobial agents (Davies 2003). One of the most ubiquitous and dangerous pathogen able to form biofilm is *Salmonella* spp.; in its sessile form, this pathogen may have important health and economic consequences, since it can serve as a potential source of contamination for food products and lead to food spoilage, reducing products' shelf life (Speranza *et al.* 2011). There are basically three different strategies in tackling biofilm problems: (1) disinfection "in time," before a biofilm develops; (2) inhibition of attachment by selecting surface materials that do not promote attachment; (3) disinfection of biofilms using harsh sanitizers. Depending on the nature of the biofilm, different sanitizers may be useful; however, the best sanitizer for a specific biofilm still has to be determined under practical conditions.

Chemical method to control biofilm is a very popular approach in food processing and food service operations due to its cost effectiveness and high efficiency. Various sanitizers available in the food industry include chlorine compounds (such as liquid chlorine, hypochlorites, chlorine dioxide, etc.), iodine and bromide compounds, quaternary ammonium compounds, organic acids (such as acetic, peroxyacetic, lactic, propionic and formic acid), peroxy acid, mixed peroxy acid organic acid, hydrogen peroxide and ozone (Simoes *et al.* 2010). Among them, an alkaline solution, a peroxygen sanitizer and a quaternary ammonium compound were selected in this study, based on their commonality and availability.

The alkaline treatments containing soda are generally used as sanitizers in disinfection processes to eliminate carbonized sediment, oil or grease, thanks to their ability to facilitate protein denaturation and fat saponification. They also have bactericidal activity causing damage to the outer membrane, ribosomes, proteins and DNA (Chavant *et al.* 2004). The bactericidal effect of this compound on biofilm cells could be attributed to the dissolution of the polymeric substances involved in the binding of cells to surfaces. Another hypothesis resulted from the capacity of the alkaline solution to diffuse throughout the protective glycocalix, to penetrate the cell wall, to disrupt the cytoplasmic membrane and to disturb the ionic gradient. The fast intracellular pH increase induces a saponification of the membrane's lipids and an inhibition of the membrane's energy producing ability. Consequently, the energy production decreases and the bacterial growth is stopped (Vasseur *et al.* 2001). Nevertheless, in this study treatment with alkaline solutions was not able to eliminate more than 82–83% of 7-days sessile cells population of *Salmonella* spp. on stainless steel surfaces. On the contrary, our results highlighted that solutions with peracetic acid were more effective than ones containing soda; in fact, the treatment with peracetic acid assured the total removal of sessile cells from stainless steel surfaces and it was effective at a low temperature. In particular, it was possible to achieve the total biofilm elimination using a sanitizing solution not much concentrated (250–800 ppm) and at a not very high temperature (45C). Peracetic acid is an ideal antimicrobial agent due to its high oxidizing potential. It is highly effective against a broad range of microorganisms and because of its effectiveness against bacteria, fungi and viruses, peracetic acid is very used as a disinfectant in the food and medical industries (Martin-Espada *et al.* 2014). In addition, this agent decomposes into safe and environmental friendly residues (acetic acid and hydrogen peroxide), hence it can be applied without rinsing risks for food contamination by toxic residues and its efficacy is not affected by protein residues (Souza *et al.* 2014).

The results also pointed out a remarkable efficacy of QAC even at very low concentrations: working at 50C, in fact, it was sufficient a poor concentrated solution (6 ppm) applied for 25 min to ensure the total biofilm elimination. Solutions of QAC are commonly used in disinfection processes; these agents are hydrophilic, cationic molecules, effective against gram positive and gram negative bacteria, moulds, and yeast, and their activity is unaffected by organic load. They are noncorrosive, nonirritating and so are often recommended for floors, walls and storage containers, surfaces which can be sanitized for long contact times, and for surfaces that do not require rinsing before production (nonfood contact surfaces) (Chmielewsky and Frank 2003). It was reported that QAC readily adsorb to bacterial surface, which is hydrophilic and negatively charged, penetrate the cell

wall, and disrupt the cytoplasmic membrane (Chmielewsky and Frank 2003). It is important noting that we tested QAC compound at very low concentrations and probably this compound could ensure the same results at lower temperatures if used at higher amount.

In 2013, Nguyen *et al.* evaluated the resistance of *Salmonella Typhimurium* biofilm to three different sanitizer treatments; in particular, the compounds tested were a quaternary ammonium salt (QAC, 200 ppm), a solution containing mixed peroxy acid/organic acid (PAO, 0.1%) and a solution with chlorine (50 ppm) for different periods of time, ranging from 0.5 to 7 min. The initial population of *S. Typhimurium* in biofilm was about, 7.86 log CFU/mL. The results recovered by these authors are in agreement with our findings, highlighting the possibility to remove over 63% of biofilms during the application of solutions containing PAO and QAC for 5 min. Treatment of QAC on biofilm for 7 min removed completely biofilms, such as the treatment with chlorine able to completely inactive *S. Typhimurium* biofilm with 5 min exposure. Ramesh *et al.* (2002) investigated the efficiency of 13 commercial disinfectants, including sodium hypochlorite and QAC-containing disinfectants, on *Salmonella* biofilm on galvanished steel and reported similar results. Contrarily, Moretro *et al.* (2009) compared nine commercial disinfectants at recommended user-concentration against 2-day-old *Salmonella* biofilms and observed that hypochlorite had the lowest effect, QAC containing disinfectants had intermediate effect while peracetic acid-containing agents and organic acids were the most effective.

Two disinfectants based on amphoteric surfactants and chlorine compounds were evaluated by Craveiro *et al.* (2015) regarding the capacity to eradicate aeromonads' preformed biofilm. Chlorine compounds were found totally efficient in removing aeromonads preformed biofilms from stainless steel, both at 4 and 20C, while surface active agents were not able to remove biofilms.

In a recent study proposed by Amaral *et al.* (2015), the effects of carvacrol and thymol against *Salmonella* spp. biofilm on polypropylene were evaluated by quantifying sessile cells during and after biofilm formation. During biofilm formation, carvacrol and thymol, at subinhibitory concentrations, reduced bacterial counts about 1–2 log, while established *Salmonella* spp. biofilms were reduced about 1–5 log. These compounds were able to reduce biofilms on polypropylene, but did not eliminate adhered cells. On the contrary, another natural compound (citric acid) was found as an effective alternative disinfectant in controlling biofilm formation in the dairy industry (Akbas and Kokumer 2015); these authors investigated the effects of 2 and 10% citric acid after 20-min exposure on biofilm formed by *Staphylococcus aureus* strains isolated from raw milk samples and compared the effects with those obtained with a commercial disinfectant containing peracetic acid at 0.3%. The removal of biofilms using peracetic acid for 20 min was on the order of 20–50%, less effective than citric acid treatments which could be proposed as valid alternatives as new natural biofilm control strategies. In other previous works, organic acids were shown as inhibitors of many pathogenic bacteria including *E. coli* and *L. monocytogenes* (Akbas and Olmez 2007), *Salmonella Gaminara* (Eswaranandam *et al.* 2004) and *Salmonella Enteritidis* (Anang *et al.* 2007) and *S. aureus* (Scannell *et al.* 1997). The inhibitory effect of organic acids was attributed to several factors such as pH depression and the ratio of undissociated forms of the acid entering into bacteria to inhibit metabolic activities and chain length (Jay *et al.* 2005).

Such disagreements recovered into the studies present about this topic, could be attributed to the variation in experimental conditions such as sanitizer concentration, exposure time and biofilm ages, thus making very difficult to compare results of studies done under different conditions.

CONCLUSIONS

The obtained results in this study suggested that peracetic acid and quaternary ammonium were the best sanitizers to remove *Salmonella* spp. biofilms from stainless steel surfaces. In particular, peracetic acid solutions assured total removal of sessile cells at a low exposure temperature, too. Unfortunately, knowledge gained from this experience with *Salmonella* spp. biofilm cannot be transferred to other types of biofilms because each biofilm is different. Thus, further investigations will be necessary in order to find standardized disinfection systems able to remove and kill all types of biofilm. In addition, further research on the factors affecting the resistance of *Salmonella* spp. biofilms against sanitizers may be also useful into the development of new sanitation strategies, which will selectively target the most resistant cells.

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