ACTION OF PERACETIC ACID ON BIOFILMS FORMED ON SURFACES USED IN FOOD INDUSTRY

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Abstract

The objective of this study was to evaluate the resistance of Bacillus subtilis biofilms to sanitizing agents under laboratory conditions, simulating a food processing environment. Biofilms were grown on stainless steel and glass coupons, for 14 days. These biofilms were treated with commercial disinfectants (peracetic acid). The testing was done through the successive exposure to different temperatures and different periods of time, between 1 and 30 minutes, using different concentration of disinfectant.
The aim of this study was to evaluate the resistance of cells in biofilms to peracetic acid. The results data suggest that the resistance of the treated biofilms to sanitizing agent may be due to attributes of extracellular polymeric substances and is not an intrinsic attribute of the cells in the biofilm.
Keywords: *biofilm, peracetic acid, disinfection.*

Introduction

Biofilms play an important role in food processing, sanitation, and food safety. Microorganisms capable to form biofilm, which may include harmful foodborne pathogens, secrete extracellular polymeric substances – EPS that allows them to adhere to various surfaces. Such surfaces include stainless steel and plastic that are used in food industry plants. Biofilms protect the cells that secrete them and they tend to make the cells more resistant to cleaning agents and disinfectants, thereby making removal of pathogens more difficult. With the cells embedded in a polysaccharide matrix, biofilms are highly resistant to disinfectants than planktonic cells. Therefore, the control of harmful biofilms is an important issue in food industry.

Peracetic acid is a bactericidal and sporicidal agent. It decomposes ultimately to hydrogen peroxide, acetic acid, and oxygen, which at recommended concentrations is toxicologically safe. It is considered to be a more effectively sporicide than hydrogen peroxide.

The objective of the present study was to evaluate the activity of peracetic acid against biofilms cells present on a stainless steel and glass surfaces. A commercial sanitizer, based on peracetic acid, was used to evaluate the efficiency of removal of these contaminants from a stainless steel and glass coupons specially designed for this study.

Experimental

Preparation of Glass and Stainless Steel Coupons

Stainless steel and glass coupons measuring 8cm x 8cm were used in this study. Coupons were immersed for 24 h in 1 N NaOH to remove any surface residue, rinsed with distilled water twice and then sterilized prior to use, by autoclaving at 121°C 45 for 15 minutes. Clean and sterile stainless steel and glass coupons were uniformly assigned with 1 cm^3 of sterile milk, and exposed to the room temperature for 14 days for biofilm forming.

Preparation of mark tests

After 14 days of biofilm formation, coupons were rinsed with 100 ml of distilled water to remove unattached cells. After that, the side of the coupon in contact with the product was repeatedly scraped about 120 times by using a sterile spatula in order to recover attached cells (Jeong, 1994).

The cells were placed in 50cm^3 of sterile physiological serum and the resulting attached cell suspension was thoroughly shaken and decimal dilutions were immediately prepared with distilled water. Dilutions were plated using agar medium and incubated at 30°C for 48 h. After the incubation period, colonies were enumerated.

Effect of exposure time of biocide against Bacillus subtilis biofilms formed on stainless steel and glass coupons

Stainless steel and glass coupons were submerged in 30 ml of each test solution for 1 minute, 5 minutes, 15 minutes and 30 minutes contact times. Immediately after exposure to each test solution, each coupon was repeatedly scraped by using a sterile spatula in order to recover attached cells (Jeong, 1994).

The cells were placed in 50cm^3 of sterile physiological serum and from resulting attached cell suspension, decimal dilutions were immediately prepared with distilled water. Dilutions were plated using agar medium and incubated at 30°C for 48 h. After the incubation times, colonies resulted were enumerated.

Effect of exposure time of disinfectant complex against Bacillus subtilis biofilms formed on stainless steel and glass coupons

Disinfectant complex (hydrogen peroxide/peracetic acid) was tested at 20°C for 1 minutes, 5 minutes, 15 minutes, and 30 minutes of contact times. The disinfectant complex was obtained mixing 0.8 % peracetic acid with hydrogen peroxide in 2:1 proportion.

Results and Discussions

Initial biofilm population on stainless steel and glass coupons prior treatment with biocides was 7.2 x 10^4 CFU/ml for stainless steel and 6.7 x 10^4 CFU/ml for glass.

The concentrations tested were 0.8% and 1.62% peracetic acid and a combination of peracetic acid and hydrogen peroxide in proportion of 2:1. Results of the decontamination tests with peracetic acid at different concentration are summarized in Figure 1 and Figure 2. Significantly bacteria cells count reductions from 7.2 x 10^4 CFU/ml (stainless steel) and 6.7 x 10^4 CFU/ml (glass) to 1.6 x 10^2 CFU/ml, respectively 1.5 x 10^4 CFU/ml were observed for 0.8 % peracetic acid treatments, after 1 minute of exposure. Values approximately were obtained for 1.62% peracetic acid, at 1 minute of exposure. After 15 minutes contact time with 0.8% peracetic acid, the number of surviving cells was reduced considerably, approximately 88.75% of cells were destroyed, and for 1.62% concentration the number was reduced with 93%, for stainless steel coupons. Similar results were obtained for glass coupon. After 30 minutes contact time with 1.62% peracetic acid, the surviving cells were destroyed in proportion of 99%, for stainless steel and 100% for glass coupons.

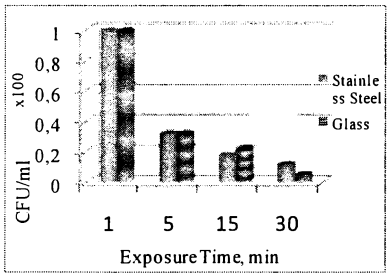

Fig. 1: Action of 0.8% peracetic acid on stainless steel and glass coupons with a 14 h biofilm after 1, 5, 15, 30 minutes of contact

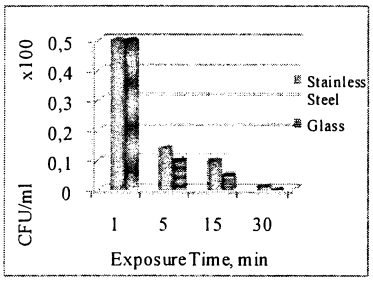

Fig. 2: Action of 1.62% peracetic acid on stainless steel and glass coupons with a 14 h biofilm after 1, 5, 15, 30 minutes of contact

In the case of disinfectant complex the activity of test solution was investigated against *B. subtilis* biofilm on stainless steel and glass coupons for 1 minute, 5 minutes, 15 minutes and 30 minutes contact times. Results are shown in Figure 3. The cell population on stainless steel coupons after 1 minute of exposure of disinfectant complex was 0.98 x 10^2 CFU/ml and for glass the cell population was reduced from 7.2 x 10^4 CFU/ml to 0.9 x 10^2 CFU/ml. After 15 minutes and 30 minutes of disinfectant complex action against biofilm cells, those were completely destroyed.

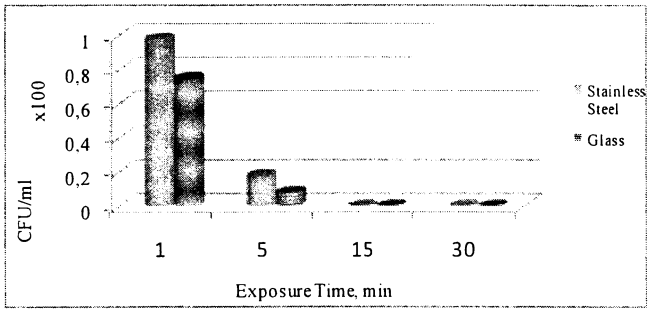

Fig. 3: Action of disinfectant complex: peracetic acid and hydrogen peroxide on stainless steel and glass coupons with a 14 h biofilm after 1, 5, 15, 30 minutes of contact

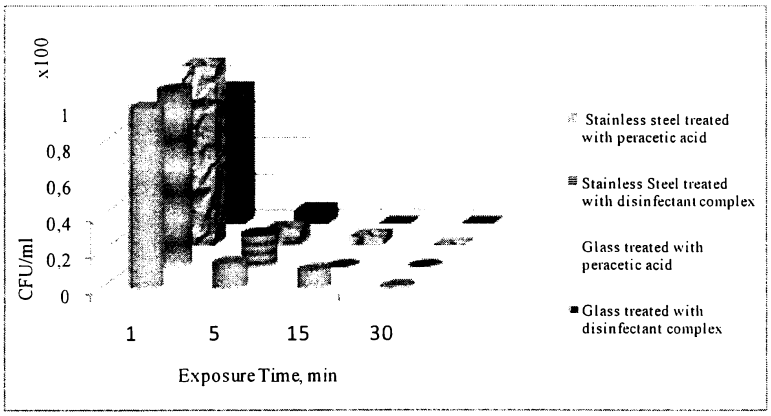

Fig. 4: Effect of exposure time of peracetic acid (1.62%) and disinfectant complex (ratio 2:1) against *B. subtilis* biofilm on stainless steel and glass coupons

Figure 4 presents an analogy between stainless steel coupons treated with peracetic acid, stainless steel coupons treated with disinfectant

complex, glass coupons treated with peracetic acid and glass coupons treated with disinfectant complex.

It is noticed that with the adding of the hydrogen peroxide, decreases the concentration of the disinfectant necessary to obtain the complete destruction of the microorganisms.

Results show that cells were highly sensitive to exposure to peracetic acid and their destruction was rapidly obtained by using a disinfectant complex. The resistance was dependent on the surface on which the cells adhered to and the exposure time of disinfectant.

Conclusions

The aim of this study was to investigate the ability of bacteria to form biofilm on different surfaces (glass and stainless steel) and the sanitizer's efficiency on biofilm removal.

Stainless steel and glass were used, because of their common use in food-processing.

Based on the results, it can be concluded that Bacillus subtilis can survive on food contact surfaces forming a biofilm and such adherent cells may not be completely removed during the washing and sanitizing processes, unless special attention is paid to the removal of biofilms. We must use the right doze of disinfectant, the best disinfectant combination and the correct time of disinfectant exposure.

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