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THE INFLUENCE OF TEMPERATURE AND MATERIAL TYPE IN BIOFILM FORMATION IN FOOD INDUSTRY

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Abstract

Microorganisms are forming structurally complex multicellular communities (biofilms) by production of an extracellular matrix. The development of biofilm in food processing area determines microbial contamination of processed food. This biofilm may contain spoilage microorganisms or/and pathogenic microorganisms. This work presumes the study of microbial adhesion on different material used in food processing. The experiments are developing within a 20 day period. Adhesion was analyzed at variable temperature (8ºC, 20ºC, 37ºC), the result being formation of a mature biofilm. We used different coupons of food-use approved materials (wood, glass, stainless steel, and polytetrafluoroethylene). We analyzed microorganism adhesion, as well as time and temperature influence on biofilm formation.
***Keywords**: biofilm, microbial adhesion*

Introduction

Biofilm formation consists of initial attachment, microcolony and EPS (extracellular polymeric substances) production, followed by maturation (Davey, 2000). The ability of microorganisms to form biofilms has been well documented. Bacterial cells make a transition from a planktonic state to a sessile state, develop, and populate a surface. Extracellular polymeric substances (EPS) may contain polysaccharides, proteins, phospholipids, teichoic and nucleic acids, and other polymeric substances hydrated to 85 to 95% water (Costerton, 1991). EPS provide biofilm protection by concentrating nutrients, preventing access of biocides, etc.

Biofilm formation can be separated into four sequential steps: initial adherence, physical irreversible adherence that involves the production of exopolymers that fix the cells, and growth of the microorganisms.

In this paper, we analyzed the adhesion to a broad range of materials used in food-processing facilities. Stainless steel, wood, glass

and PTFE were selected because of their common use in food-processing plants and because they have different physicochemical characteristics. Adhesion to these materials was evaluated after a determinate period of time. The aim of this study was to investigate the ability of bacteria to adhere and to form biofilm on abiotic surfaces at different temperatures.

Experimental

The materials tested are wood, glass, stainless steel, and polytetrafluoroethylene. All test materials were cut into coupons (8·8cm). The coupons were sterilized at 180 ºC, for 1 hour. Like substratum we used 1 cm^3 of milk, uniformly assign on coupon's surfaces. The samples were maintained at different temperature (8ºC, 20 ºC, 37ºC) in laboratory condition for 7 days.

Each coupon was 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 50 cm^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 for biofilm formation and incubated at 30 °C for 48 h. After the incubation period, colonies were enumerated and the number of biofilm forming cells per cm^2 was calculated. The colonies resulted were selected for the identification (colour, shape, surface, diameter, margin, elevation, opacity and consistency). The selected colonies were identified by colony morphology and Gram's reaction.

Results and Discussions

Some studies have shown that bacterial attachment is more reduced on hydrophilic surfaces, like glass, comparative with other hydrophobic material, like stainless steel; for example, study cited by Hermensson (1999).

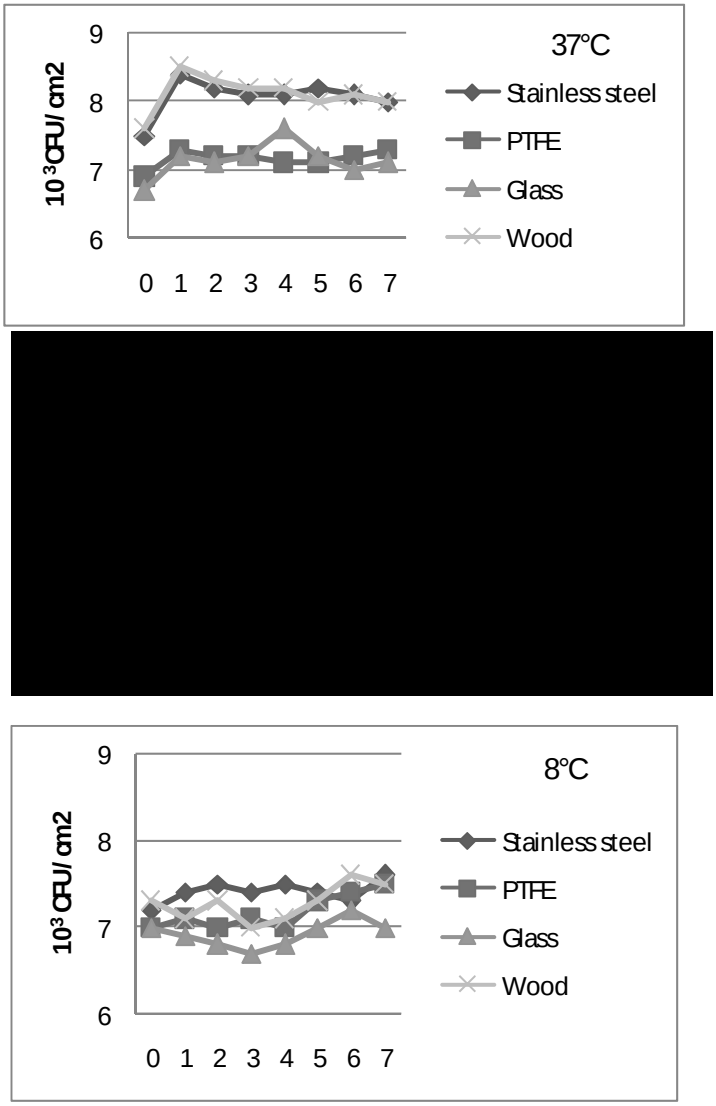

Figure 1. Biofilm growth at 8°C, 20°C, and 37°C on stainless steel, PTFE, glass and wood surfaces

The data in figure 1 show the growth of cells at 8°C, 20°C, and 37°C on stainless steel, PTFE, wood and glass surfaces. The initial adherent population was greater on stainless steel at 37°C, while it was greater on PTFE at 20°C and was equivalent on the two surfaces at

8°C. For wood and glass the microbial growth is higher at 37°C. Under all experimental conditions except on glass at 20°C and 8°C, the number of attached cells increased with time or remains constant.

The maximum number of adhering cells was reached after 1, 2, or 5 to 7 days for the stainless steel, PTFE and wood surfaces at 37, 20, and 8°C, respectively. For glass, maximum number of adhering cells was obtain after 4 days at 37°C, after 2 days at 20°C and after 6 days at 8°C. At low temperature, the colonization of surfaces was very slow, and a reduction in the adherent population was observed on glass.

The results showed that, regardless of growth temperature the stainless steel coupons were completely overlaid by the bacterial biofilm by the end of 5 days. The colonization of stainless steel coupons was rapid.

At least 80% of the coupons surface was covered by the biofilm after 12 h at 37°C and 20°C. For wood, PTFE and stainless steel, the colonization reached 100% after 24 h at 37°C, but decreased after 2 days because of detachment. For glass the colonization reached 100% after 4 days, at 37°C. At 20°C the hydrophilic surface was again completely colonized, with a maximum after 2 days; detachment begun immediately with a slow variation after 5 day. Interestingly, a phase of detachment took place after 1 day at 20°C on stainless steel, but this was transitory and the surface was almost completely colonized again 24 h later. At low temperature, the results showed that the hydrophilic surface are not intense colonized.

The percentages of surface colonized, as a function of time, are represented in Figure 2.

At 37°C wood, stainless steel and PTFE surfaces were very fast colonized by formation of bacterial support. At 20°C colonization is reduced and the detachment is more rapid for all surfaces after 5 days. At 8°C the number of cells is reduced especially for hydrophilic surfaces.

It was proposed (Cunliffe, 1999) a two-step, time-dependent model for adhesion of marine bacteria to a surface (glass). First, there was an instantaneous reversible adhesion, with only weak interactions occurring between the bacteria and the surface; this was followed by irreversible adhesion mediated by the formation of extracellular material.

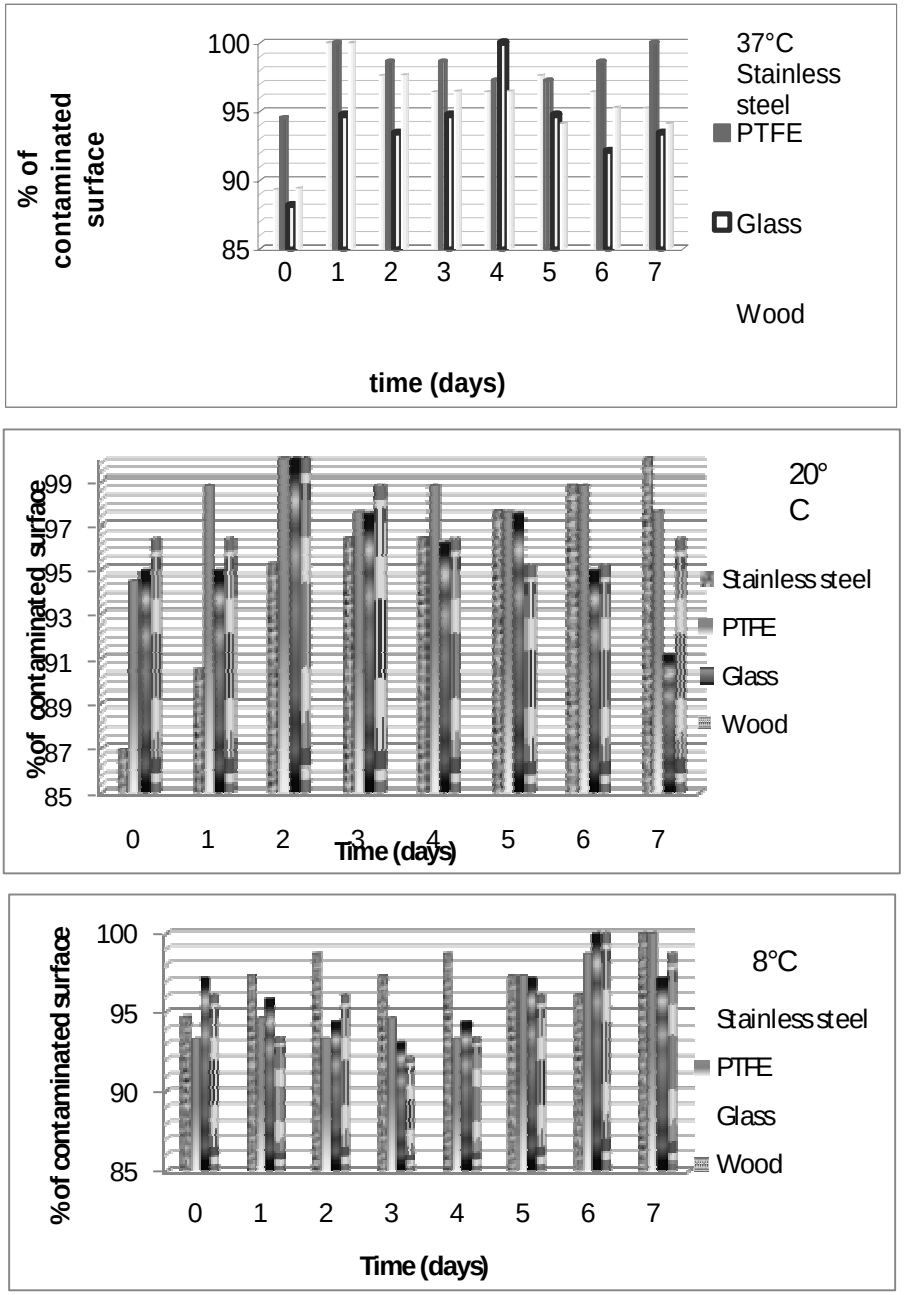

Figure 2. Percentages of surface colonized at 37°C, 20°C, and 8°C on stainless steel, PTFE, wood and glass

Conclusions

The data obtained in the present study show that the two-step model is generally true for bacteria to attach to a wide variety of materials. The bacteria completely or almost completely colonized stainless steel and PTFE surfaces, except at low temperature for PTFE and glass. From a practical point of view, our results demonstrate that the use of hydrophilic surfaces in cold rooms may minimize the development of biofilms in food plants.

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