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Evaluation of SICON® surfaces for biofouling mitigation in critical process areas

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ABSTRACT

In industrial processes, particularly in the food sector, sustainability is increasingly important. Consumers demand safer food and this is often associated with elevated cleaning costs and high environmental impacts in order to reduce contaminations on equipment and products.

Modified surfaces are seen as a promising strategy for biofouling mitigation and contamination prevention. In this work, the performance of a modified Diamond-Like Carbon (DLC) surface designated by SICON® (a-C:H:Si:O) was compared with stainless steel (316L) regarding bacterial adhesion, biofilm formation and cleanability. Assays were performed at different temperatures using *Escherichia coli*, one of the most persistent foodborne microorganisms and also the natural flora present in the water from an industrial salad washing line. Bacterial adhesion on SICON® and stainless steel were similar and favored at a higher temperature (30 °C). Biofilm formation was reduced on SICON® (1–2 Log) and this may be explained by the lower ratio between the Lifshitz-van der Waals apolar component and the electron donor component (γ^{LW}/γ^{-}) of this surface. It was also shown that after performing a cleaning treatment with chlorine, reduction of viability counts was much higher in SICON® (about 3.5 Log reduction and 15% removal) when compared to stainless steel (1.6 Log reduction and 6% removal). Additionally, it was observed that 18 h after treatment, biofilm values in SICON® were similar to those obtained with stainless steel.

Results indicate that for industries with cleaning frequencies of up to 6 h, the use of SICON® on critical areas enables operation at a much higher hygienic level.

1. Introduction

There is an increasing demand for sustainable manufacturing processes in the food industry (Del Borghi et al., 2014). A sustainable process implies an engagement between high-quality and hygienic products, low environmental impact of the process, reduced costs and lower health risks (Gomes da Cruz et al., 2015; Mauermann et al., 2009). However, this is challenging since elevated cleaning costs are incurred due to the use of disinfectants, water and energy in order to reduce contamination on processing equipment and food products (Gomes da Cruz et al., 2015; Mauermann et al., 2009). Moreover, the use of chemical disinfectants and high water consumption have an elevated environmental impact (Moreira et al., 2014b). The unavoidable attachment of bacterial cells on industrial surfaces and further biofilm

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development is at the core of the problem (Simões et al., 2010). Bacteria within a biofilm are protected by a self-produced matrix composed by extracellular polymeric substances (EPS). This matrix protects the cells from chemical disinfectants, biocides, surfactants and from mechanical forces promoted by water jets and by scrubbing and scraping actions (Simões et al., 2009). Moreover, in industrial plants, there are critical zones such as crevices, corners, joints, valves, which are difficult to clean due to reduced access and where lower fluid velocities may be found, making these zones suitable niches for biofilm accumulation and growth (Lemos et al., 2015a,b). In these zones, higher amounts of disinfectants and water have to be used in order to achieve recommended cleaning standards and this has environmental and economic impacts.

In food processes such as in the dairy industry, microorganisms may be detected on surfaces after 2 h and values of 8.55×10^4 cells cm^{-2} can be reached after 5 days (Holah and Kearney, 1992). On a fish fileting process, values of 3.35×10^3 cells cm^{-2} were found after 6 h of operation and in baked beans transport belts, values higher than 4.30×10^7 cells cm^{-2} can be achieved after 16 h (Holah and Kearney, 1992).

Escherichia coli is one of the most persistent foodborne microorganisms (Dourou et al., 2011; Sagong et al., 2011; Shi and Zhu, 2009) and its presence on food-contact surfaces has been associated with its ability to attach and form biofilms on these surfaces (Dourou et al., 2011). The most widely used method to detect the presence of the biological contaminants on the equipment surfaces is by swabbing and bacterial cultivation in order to determine the number of cells per cm^2 (Sudheesh et al., 2013). Additionally, the microbiological load can also be measured by the adenosine triphosphate (ATP) level (Sudheesh et al., 2013). Both methods only detect viable microorganisms that can grow during the cultivation step or produce ATP. However, it is known that a biofilm is composed by EPS, viable bacteria (the so-called "active layer") and by non-viable bacteria that are usually located at the bottom of the biofilm (Vieira and Melo, 1999). Therefore, the standard methods used to detect attached bacteria on industrial food contact surfaces does not take into account the non-viable bacterial layer and the EPS that may sometimes represent the majority of the biofilm. Thus, after equipment sanitizing, the traditional methods used to determine the cleaning efficiency are not taking into account the non-living biofilm components that may have an important role on biofilm regrowth.

The modification of energetic and topographic surface properties is seen as a good strategy for fouling mitigation (Mauermann et al., 2009) despite the additional costs of surface preparation (Gomes da Cruz et al., 2015). These modifications are excepted to delay bacterial adhesion and/or facilitate the cleaning processes (Mauermann et al., 2009). SICON® is a Diamond-Like Carbon (DLC) coating (a-C:H:Si:O), approved as a food contact surface that has been investigated as alternative to stainless steel in food manufacturing plants due its thermal conductivity, low friction, smoothness, wear resistance and anti-fouling properties (Boxler et al., 2013a). Boxler et al. (2013a,b) investigated the performance of SICON® and other DLC coatings against milk fouling (whey protein and milk salts). Results showed that surface modification directly affected the formation of deposits, their composition, as well as their adhesive strength and that this was due to the electron donor component of the surface energy. They concluded that a lower deposit mass was formed on SICON® compared to stainless steel and that this surface was easier to clean. Saikhwan (2013) made a preliminary study with DLC coatings in order to evaluate their suitability for biofouling mitigation in building exteriors. Fluid dynamic gauging was used to determine the thickness and the shear stress (between 1.5 and 8 Pa) required to clean biofilms of *Pseudomonas fluorescens* and *Arthronema africanum* formed on the selected surfaces. Although no conclusive results were obtained with SICON®, it was suggested that surface energy had little influence on biofilm formation. Additionally, it was observed that surface roughness affected biofilm formation but had negligible effects on biofilm cleaning.

Despite the beneficial effects of SICON® in the mitigation of abiotic fouling, the preliminary results with biological fouling were inconclusive. Additionally, to the best of our knowledge, this surface has never been evaluated in microbial fouling mitigation in industrial conditions. In this work, the performance of SICON® and stainless steel were compared regarding *E. coli* adhesion, biofilm formation and cleaning. Assays simulated industrial settings using process water from a salad washing line and also tested some extreme operational conditions (higher temperature and nutrient composition) to evaluate if the use of this modified surface in critical areas could be beneficial in maintaining a higher hygienic level in different industrial plants. Biofilm quantification was made by viable plate counting and by biofilm thickness measurement. These methods enabled the determination of the viable biofilm amount and the total biofilm amount. The importance of these measurements on the evaluation of CIP (cleaning in place) efficiency on food industries is discussed.

2. Material and methods

2.1. Bacterial and culture conditions

Escherichia coli JM109(DE3) from Promega (USA) was used in this study because it has shown a good biofilm forming ability in a variety of *in vitro* platforms operated at different shear stresses (Moreira et al., 2013, 2014a; Teodósio et al., 2012). Additionally, it was shown that its biofilm formation is similar to other *E. coli* strains which are often used for antimicrobial susceptibility and disinfection tests (Gomes et al., 2014). A starter culture was obtained by inoculation of 500 μL of a glycerol stock (kept at −80 °C) to a total volume of 200 mL of inoculation medium with 5.5 g L^{-1} glucose, 2.5 g L^{-1} peptone, 1.25 g L^{-1} yeast extract in phosphate buffer (1.88 g L^{-1} KH_2PO_4 and 2.60 g L^{-1} Na_2HPO_4) at pH 7.0, as described by Teodósio et al. (2011). This culture was grown in a 1 L shake-flask, incubated overnight at 30 °C with orbital agitation (120 rpm). A volume of 150 mL of this culture was used for the adhesion assays, and a volume of 50 mL was used to inoculate the intermediate fermenter used for the biofilm assays.

2.2. Surface preparation

Round coupons (1 cm of diameter) made from electropolished stainless steel (AISI 316L/X2CrNiMo17-12-2/1.4404) and SICON® were tested. The coatings were prepared by the Fraunhofer Institute for Surface Engineering and Thin Films (IST) in Braunschweig, Germany using the PECVD method (PECVD: Plasma enhanced Chemical Vapor Deposition). A detailed description of the SICON® preparation method was disclosed before (Corbella et al., 2009; Grischke et al., 1998).

Coupons were first scrubbed and disinfected with ethanol (70%) and were then immersed in a commercial bleach