Evaluation of SICAN performance for biofouling mitigation in the food industry

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Abstract

Biological fouling in food industry leads to an increase in maintenance costs, decreases operational efficiencies and promotes food contamination leading to economic losses and the dissemination of foodborne pathogens. In order to maintain production efficiency and hygienic standards, cleaning in place (CIP) procedures are required. However, the existence of critical zones shielded from the main flow carrying the CIP disinfectants requires new strategies for reducing biofilm buildup and/or easy to clean surfaces. In this work, a Diamond-Like Carbon (DLC) coating modified by incorporation of silicon (a-C:H:Si or SICAN), was evaluated regarding bacterial adhesion, biofilm formation and cleanability. Assays included the natural flora present in industrial water (from a salad washing line) and *Escherichia coli*, one of the most persistent foodborne microorganisms. Results show that bacterial adhesion and biofilm formation on SICAN and stainless steel were similar, thus surface modification was not able to prevent biological fouling development. However, it was verified that after performing a cleaning protocol with chlorine, reduction of bacterial counts was much higher in SICAN (about 3.3 Log reduction) when compared to stainless steel (1.7 Log reduction). Although full biofilm recovery was observed on both surfaces 18 h after treatment, an operational window was identified for which processes with cleaning intervals of about 6 h could potentially use SICAN surfaces on critical areas (such as dead zones, crevices, corners, joints) and therefore operate at a much higher hygienic level than the one attained with stainless steel.

Keywords: Adhesion, Biofilm, Cleaning, *Escherichia coli*, SICAN, Stainless steel

1. Introduction
Biological fouling establishment in industrial piping, equipment and cooling systems is a serious problem in the food industry (Brooks & Flint, 2008). The formation of these biological deposits, starts with the interaction between planktonic (free floating) bacteria and the industrial surfaces (Srey, Jahid, & Ha, 2013). After a first contact, bacteria adhere to the surface and start producing extracellular polymeric substances. Further cell growth leads to biofilm formation (Costerton, Stewart, & Greenberg, 1999). Biofilms can cause equipment damage through corrosion, local clogging and heat transfer resistance, leading to increased maintenance costs and decreased equipment operational efficiencies (Characklis, 1981). Additionally, they can cause contamination of product and staff leading to economic losses and health incidents (Agle, 2007; Bott, 2011; Shi & Zhu, 2009).

*Escherichia coli* has been reported as one of the most persistent foodborne microorganisms (Dourou, et al., 2011; Sagong, et al., 2011; Shi & Zhu, 2009) that can be found in vegetable process industries, meat industries and ready-to-eat products (Srey, et al., 2013). The presence of *E. coli* on food-contact surfaces is well documented and has been associated with the ability of this bacterium to attach and form biofilms on these surfaces (Dourou, et al., 2011). In a biofilm, bacteria are more resistant to biocides becoming more difficult to eradicate (Simões, Simões, & Vieira, 2010). Sodium hypochlorite is the most widely used industrial disinfectant (Lomander, Schreuders, Russek-Cohen, & Ali, 2004). Despite its proven efficiency against planktonic bacteria, care must be taken when dealing with adhered bacteria (Luppens, Reij, van der Heijden, Rombouts, & Abee, 2002). Rossoni and Gaylarde (2000) isolated *E. coli, Pseudomonas fluorescens* and *Staphylococcus aureus* from chicken carcasses and studied the efficiency of sodium hypochlorite (100 and 200 ppm from a 10% active chlorine formulation) and peracetic acid (250 and 1000 ppm from a 14% active ingredient formulation) on the killing and removal of
those bacteria adhered on a stainless steel surface. In all cases, sodium hypochlorite was more
effective than peracetic acid and 1.5 to 2 Log reductions were obtained with *E. coli* (lower
reductions were obtained for the other species).

In order to maintain the production efficiency and meet hygienic standards, regular and intensive
cleaning in place (CIP) procedures have been implemented in industry (Shi & Zhu, 2009).

Cleaning times in some food industries can represent up to 15% of the total production time
(Mauermann, Eschenhagen, Bley, & Majschak, 2009) and some typical cleaning frequencies are
indicated in Table 1. These frequent stops for cleaning, as well as the energy and chemical products
used for disinfection translate into elevated costs (Mauermann, et al., 2009). Despite the progress
in the field of industrial CIP, this operation is still difficult and depends on many factors like the
nature and age of the biological fouling layer, the cleaning agent composition and concentration,
the degree of turbulence of the cleaning solution and the characteristics of the surface (Bremer,
Fillery, & McQuillan, 2006; Pogiatzis, Vassiliadis, Mergulhão, & Wilson, 2014). In industrial
plants it is common to find critical zones such as crevices, corners, joints, valves, which are
difficult to clean due to difficult access and where lower fluid velocities may be found, making
these zones suitable niches for biofilm accumulation and growth (Lemos, Mergulhão, Melo, &
Simões, 2015). One of the approaches to reduce biological fouling and to increase the removal of
formed deposits in these critical zones is the modification of the energetic and topographic surface
properties (Mauermann, et al., 2009) in order to reduce bacterial adhesion and to improve
cleanability (Boxler, Augustin, & Scholl, 2013b). Diamond-like carbon (DLC) coatings, approved
as food contact surfaces, have been investigated as alternative to stainless steel in food
manufacturing plants due to their thermal conductivity, low friction, smoothness, wear resistance
and anti-fouling properties (Boxler, Augustin, & Scholl, 2013a). A modification of DLC coatings
by incorporating silicon (a-C:H:Si) leads to some additional characteristics such as increased optical transmittance and thermal resistance (Corbella, Bialuch, Kleinschmidt, & Bewilogua, 2009). Some reports have shown the benefits of using modified DLC coatings on abiotic fouling mitigation in the food industry. Augustin, Geddert, and Scholl (2007) investigated the influence of aluminum, copper, DLC coatings, and modified DLC coatings (a-C:H:Si/SICAN and a-C:H:Si:O/SICON®) on the induction period of whey protein fouling deposition. These authors observed that the fouling induction period may be extended in an electro-polished stainless steel surface coated with SICAN. In other study (Geddert, Bialuch, Augustin, & Scholl, 2007), it was observed that this surface also extended the induction period of CaSO₄ crystallization. Boxler, et al. (2013b) investigated the influence of SICAN and SICON® coatings, against milk fouling. Results showed that surface modification directly affected the formation of deposits, their composition, as well as their adhesive strength. They concluded that SICAN was effective for abiotic fouling mitigation. Later, these authors validated their results in a pilot-scale plate heat exchanger and concluded that SICAN is a good alternative to stainless steel to be applied on heat transfer surfaces in the food industry (Boxler, Augustin, & Scholl, 2014). Additionally, Boxler, et al. (2013a) also verified in another study that cleaning of whey protein and milk salts is easier on SICAN than on stainless steel.

Despite these promising evidences showing the beneficial effects of SICAN in the mitigation of abiotic fouling, no studies were performed concerning bacterial fouling in industrial conditions. In this work, the behavior of a-C:H:Si/SICAN coatings and stainless steel were compared regarding E. coli adhesion, biofilm formation and cleaning. Assays tried to replicate industrial settings using process water from a salad washing line and also testing some extreme operational conditions (higher temperature and contact times) to evaluate if the use of this modified surface in critical
areas could be beneficial in maintaining a higher hygienic level in industrial plants.

2. Material and methods

2.1. Bacteria 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., 2014; Moreira, et al., 2013; Teodósio, Simões, Alves, Melo, & Mergulhão, 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 ºC) 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 KH₂PO₄ and 2.60 g L⁻¹ Na₂HPO₄) 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 ºC with orbital agitation (120 rpm). A volume of 100 mL of this culture was used for the adhesion assays described in section 2.4. A volume of 50 mL of this culture was used to inoculate the intermediate fermenter used for the biofilm assays described in section 2.5.

2.2. Surface preparation

Round coupons (1 cm of diameter) made from electro-polished stainless steel (AISI 316L/X2CrNiMo17-12-2/1.4404) and SICAN coated coupons were tested. The coatings were prepared by the Fraunhofer Institute for Surface Engineering and Thin Films (IST) in Braunschweig, Germany and a detailed description of the SICAN preparation method was disclosed before (Corbella, et al., 2009; Grischke, Hieke, Morgenweck, & Dimigen, 1998).
Surfaces were cleaned with ethanol and then immersed in a commercial bleach (Continente, Portugal) solution (0.2% v/v) for 20 min under strong agitation. To remove the bleach, coupons were aseptically rinsed and washed again with sterile distilled water under strong agitation, for 20 min.

### 2.3. Surface characterization

Surface hydrophobicity was evaluated considering the Lifshitz-van der Waals acid base approach (van Oss, 1994). The contact angles were determined automatically by the sessile drop method in a contact angle meter (OCA 15 Plus; Dataphysics, Filderstadt, Germany) using water, formamide and α-bromonaphtalene (Sigma) as reference liquids. The surface tension components of the reference liquids were taken from literature (Janczuk, Chibowski, Bruque, Kerkeb, & Gonzales-Caballero, 1993). For each surface, measurements with each liquid were performed at 25 ± 2 °C.

The model proposed by van Oss (1994) indicates that the total surface energy ($\gamma_{\text{Tot}}$) of a pure substance is the sum of the Lifshitz-van der Waals components of the surface free energy ($\gamma_{\text{LW}}$) and Lewis acid-base components ($\gamma_{\text{AB}}$):

$$\gamma_{\text{Tot}} = \gamma_{\text{LW}} + \gamma_{\text{AB}}$$  \hspace{1cm} (1)

The polar AB component comprises the electron acceptor $\gamma^+$ and electron donor $\gamma^-$ parameters, and is given by:

$$\gamma_{\text{AB}} = 2\sqrt{\gamma^+ \gamma^-}$$  \hspace{1cm} (2)

The surface energy components of a solid surface (s) are obtained by measuring the contact angles ($\theta$) with the three different liquids (l) with known surface tension components, followed by the simultaneous resolution of three equations of the type:
The degree of hydrophobicity of a given surface is expressed as the free energy of interaction ($\Delta G$ mJ.m$^{-2}$) between two entities of that surface immersed in a polar liquid (such as water (w) as a model solvent). $\Delta G$ was calculated from the surface tension components of the interacting entities, using the equation:

$$\Delta G = -2\left(\sqrt{\gamma_{s}^{LW} - \gamma_{w}^{LW}}\right)^2 + 4\left(\sqrt{\gamma_{s}^{-}w^{+}} + \sqrt{\gamma_{s}^{+}w^{-}} - \sqrt{\gamma_{s}^{+}w^{+}} - \sqrt{\gamma_{s}^{-}w^{-}}\right).$$

If the interaction between the two entities is stronger than the interaction of each entity with water, $\Delta G < 0$ mJ.m$^{-2}$, the material is considered hydrophobic, if $\Delta G > 0$ mJ.m$^{-2}$, the material is hydrophilic.

2.4. Adhesion assays

The adhesion assays were made in three different media, an industrial water collected from a salad washing line (with a bacterial load of $3.3 \times 10^5$ CFU mL$^{-1}$ and a Chemical Oxygen Demand below 26 mgO$_2$ L$^{-1}$), the same industrial water spiked with *E. coli* JM109(DE3) and a low nutrient medium containing 0.055 g L$^{-1}$ glucose, 0.025 g L$^{-1}$ peptone, 0.0125 g L$^{-1}$ yeast extract in phosphate buffer (1.88 g L$^{-1}$ KH$_2$PO$_4$ and 2.60 g L$^{-1}$ Na$_2$HPO$_4$) at pH 7.0 (a 1:100 dilution of the inoculation medium) also spiked with *E. coli* JM109(DE3). For *E. coli*, two aliquots (50 ml) from the overnight grown culture (described in section 2.1) were used to harvest cells by centrifugation (10 min, 3,202 g). Cells were washed twice and then resuspended in the final medium. For the low nutrient medium, an appropriate volume was used to reach a final optical density (OD) of 0.1 at 610 nm, which corresponds to $7.60 \times 10^7$ cell mL$^{-1}$. The same volume was used for the industrial water and a total bacterial load of $7.63 \times 10^7$ cell mL$^{-1}$ was obtained.
To conduct the adhesion assays, a volume of 4 mL of the industrial water and the *E. coli* inoculated suspensions was transferred into separate wells of a sterile 6-well polystyrene, flat-bottomed microtiter plate (VWR Internacional, Portugal) containing a single coupon of the tested materials (stainless steel or SICAN). The microtiter plates were incubated under shaking conditions in order to obtain an average shear stress of 0.25 Pa (Salek, Sattari, & Martinuzzi, 2011). This shear stress can be found in critical zones (corners, valves, angles, pumps, etc) in industrial plants (Cunault, et al., 2015; Jensen & Friis, 2005; Lelièvre, et al., 2002; Liu, et al., 2006). The adhesion assays with the industrial water (with and without the *E. coli* spike) were conducted at 5 ºC in order to mimic the industrial conditions found at the salad washing facilities (Figure 1). The low nutrient medium was also used in this work for comparison purposes and, in order to assess the effect of temperature on bacterial adhesion, assays were performed at 5 ºC and 30 ºC. It was found that *E. coli* adhesion (during 2 h) was similar at these temperatures on both surfaces (see supplementary material, Figure S1). However, it has been reported that temperatures above 18 ºC and below 55ºC can potentiate microbiological development and food deterioration, (Garayoa, Díez-Leturia, Bes-Rastrollo, García-Jalón, & Vitas, 2014; Kim, Yun, Lee, Hwang, & Rhee, 2013; Kuo & Chen, 2010). Therefore, a temperature of 30 ºC was selected to conduct the biofilm formation and cleaning assays (Figure 2) in order to test these surfaces under the worst possible conditions.

At different sampling times, between 0.5 and 6 h, coupons were removed from the wells and rinsed with sterile saline to remove loosely attached cells. Total cell counts were obtained by direct staining with 4′,6-diamidino-2-phenylindole (DAPI) due to the high sensitivity of the method, as previously described by Lemos et al. (2014). Cells were visualized under an epifluorescence microscope (Eclipse LV100, Nikon, Japan) equipped with a filter block sensitive to DAPI fluorescence (359-nm excitation filter in combination with a 461-nm emission filter). For each
coupon, a minimum of 10 fields were counted and the results were expressed as logarithm of the number of attached cells per cm². Three independent experiments were performed for each surface and medium.

2.5. Biofilm formation and sampling

A flow cell system (see supplementary material, Figure S2) was used for these assays and it is composed by a recirculating tank, one vertical flow cell, peristaltic and centrifuge pumps and one intermediate fermenter (Teodósio, et al., 2011). This fermenter contained initially 375 mL of sterile inoculation medium and was inoculated with 50 mL of the overnight culture (described in section 2.1). After inoculation, it was fed (14.5 mL h⁻¹) with sterile inoculation medium. The culture was then left to grow under agitation (with a magnetic stirrer) during 4 h at room temperature and aerated using an air pump (air flow rate 250 L h⁻¹). After this time, the culture was used to continuously inoculate (0.025 L h⁻¹) the recirculating tank of the flow cell system, initially containing 1.5 L of saline solution (8.5 g L⁻¹ NaCl). The recirculating tank was also fed (300 mL h⁻¹) with the sterile low nutrient medium used in the adhesion assay described in section 2.4 (1:100 dilution of the inoculation medium). Biofilms were formed on SICAN or stainless steel coupons, cleaned as described in section 2.2 and placed in the vertical flow cell. The bacterial suspension was circulated in the system at a flow rate of 300 L h⁻¹ in order to obtain a shear stress of 0.25 Pa (Teodósio, et al., 2013). Temperature was kept at 30 °C and biofilm formation was monitored for five days. For biofilm sampling (Teodósio, et al., 2011), the system was stopped to allow coupon removal and carefully started again maintaining the flow conditions described above. Coupons were removed daily (24 h step) from the flow cell (and replaced by new ones to seal the system).
and the number of CFU per mL was determined by viable plate counting using PCA (plate counting agar). Three independent experiments were performed for each surface.

2.6. Cleaning and regrowth

After the five days of biofilm formation, the intermediate fermenter was disconnected from the recirculating tank and the flow cell system was emptied. A disinfection solution of 0.2% commercial bleach (Continente, Portugal) was then applied and recirculated (at 300 L.h⁻¹) in the system for 20 min. The system was then emptied and filled with sterile water that was recirculated for an additional 20 min in order to remove the disinfectant from the system. The water was then removed and the system was filled with fresh sterile low nutrient medium (1:100 dilution of the inoculation medium) and the recirculation restored maintaining the same flow conditions. The coupons with biofilm formed during 5 days were analyzed immediately after the disinfection process and after 6 h, 18 h and 24 h in order to determine the number of CFU per mL. The amounts of removed biofilm were assayed by wet weight determination by weighing the coupons containing biofilms prior and after disinfection as described before (Teodósio, et al., 2011). Three independent experiments were performed for each surface.

2.7. Statistical analysis

Paired t-test analyses were performed to evaluate if statistically significant differences were obtained with the two materials. Three independent experiments were performed for each surface and medium. Each time point was evaluated individually using the three independent results obtained with stainless steel and the three individual results obtained with SICAN. Results were considered statistically different for a confidence level greater than 95% ($P < 0.05$). Standard
deviation between the 3 values obtained from the independent experiments was also calculated.

3. Results and Discussion

Surface properties of SICAN and stainless steel were first determined and the results are presented in Table 2. From the total free energy results it is possible to observe that both surfaces are hydrophobic ($\Delta G < 0$ mJ m$^{-2}$). Regarding $\gamma^-$ and $\gamma^+$, results showed that both surfaces are monopolar electron donors. Additionally, it can be observed that the Lifshitz-van der Waals component $\gamma^{LW}$ contributed more significantly to the total surface energy $\gamma^{Tot}$ than the acid-base polar component $\gamma^{AB}$. It was also possible to verify from the literature (Boxler, et al. 2013a) that both surfaces have a similar roughness.

Figure 1 shows the cell adhesion results on both surfaces at 5 ºC. Similar adhesion results were obtained on both surfaces using industrial water (figure 1a). Moreover, adhesion did not increase with time (from 0.5 to 6 h). These results showed that microorganisms belonging to the natural flora present in the industrial water are capable of adhering to both surfaces equally. Furthermore, addition of another microorganism in significant amounts (2.3 Log difference) did not potentiate cell adhesion (figure 1b) indicating that adhesion was not affected by planktonic cell concentration in any of the surfaces ($P > 0.05$). This was further confirmed by the results in the low nutrient medium (figure 1c) which were similar to the ones obtained in the previous conditions ($P > 0.05$). Thus, it seems that under the conditions tested, cell adhesion was not strongly influenced by planktonic cell concentration, surface type and composition of the culture medium (Figure 1) and also assayed temperature (Figure S1). In a study by Azevedo, Pinto, Reis, Vieira, & Keevil (2006) with *Helicobacter pylori*, temperatures between 4 ºC and 37 ºC were tested and it was also observed that there was no effect of the temperature on *H. pylori* adhesion to stainless steel. The
similar adhesion values observed on both surfaces may be explained by the effect of the surface properties. Both surfaces are hydrophobic and have similar roughness (table 2) and it is known that these two parameters have an important role on microbiological adhesion (Goulter, Gentle, & Dykes, 2009; Schlisselberg & Yaron, 2013), thus in this work it seems that adhesion is being controlled by these two parameters. Boxler, et al. (2013a) performed a study with several surfaces for milk soil fouling mitigation. They also observed that a similar deposit mass was obtained on the electro-polished stainless steel and on the SICAN coating. In the present work, it was also observed that an increase in planktonic cell load did not lead to an increase in the number of adhered cells and that after initial adhesion (30 min), a cellular adhesion plateau was attained. In the study made by Azevedo, et al. (2006) the effect of the inoculum concentration on the adhesion of H. pylori to stainless steel was also evaluated. They observed that there was a maximum number of bacterial cells that could adhere to a surface after a certain elapsed time and therefore increasing the initial cellular concentration did not lead to a higher H. pylori adhesion. Cerca, Pier, Oliveira & Azeredo (2004) reached a similar conclusion when studying coagulase-negative staphylococci adhesion by a static and dynamic method. They observed that bacterial adhesion increased from 30 min to 120 min but from this time onwards the number of adhered bacteria remained constant. In a previous study with the same strain, it was found that hydrodynamics (shear stress) was controlling the thickness of an E. coli biofilm grown at two different substrate loading rates (Teodósio, et al., 2011). It was also recently shown for this strain that shear stress can affect cell adhesion and can even modulate the effects of the surface properties (Moreira, et al., 2014). Thus, it is likely that the hydrodynamic conditions were also controlling bacterial adhesion in the present study and their effect was stronger than the bacterial composition, bacterial concentration, medium composition, surface properties and assay temperature. Although it has been reported that
temperatures above 18 °C and below 55 °C can potentiate microbiological development and food deterioration, (Garayoa, et al., 2014; Kim, et al., 2013; Kuo & Chen, 2010), in the present study it was verified that *E. coli* adhesion was not influenced by temperature.

Figure 2 depicts the bacterial adhesion values obtained at 30 °C as well as the biofilm formation and cleaning results. Since cleaning intervals in the food industry vary (Table 1), biofilm formation was allowed to occur for 5 days prior to cleaning. In this figure it is possible to verify that bacterial adhesion (until 6 h) was constant with time and similar on both surfaces for all time points tested ($P > 0.05$). Regarding biofilm formation (from 24 until 120 h), it was also observed that similar values were obtained on both surfaces ($P > 0.05$). A slight increase in the number of attached cells was observed for both surfaces up to 72 h and after that the number of cells stabilized. It is well known that after the bacterial adhesion phase, the biofilm starts to grow from the adhered cells and a biofilm increase is observed until a pseudo steady state is reached. At this point, the biofilm sloughing rate equals the growth rate and the steady state is attained (Melo & Bott, 1997). In this work, this pseudo steady state was achieved at 72 h.

After 5 days of biofilm formation (120 h), the biofilm was treated with a 0.2% chlorine solution (about 100 ppm) for 20 min and after this treatment it was possible to observe that the number of viable cells (1 h after the beginning of the treatment) decreased approximately 1.7 Log on stainless steel and 3.3 Log on SICAN (Figure 2). Besides viability, the amount of biofilm assayed by wet weight determination showed that there was a 20% reduction after treatment in stainless steel whereas a 40% reduction was obtained with SICAN (data not shown). An induction period of 6 h was observed for both surfaces with a rapid bacterial growth for SICAN after this period. For the particular conditions tested on this work, there was a time window of more than 6 h (Figure 2) for which operation can proceed at a much higher hygienic status if SICAN is used (1.6 Log
difference). At 18 h after treatment, both surfaces recovered the bacterial colonization levels attained during the first 24 h of biofilm formation. Some authors (Bang, et al., 2014; Lomander, et al., 2004; Schlisselberg & Yaron, 2013) investigated the effect of surface properties on biofilm susceptibility to chlorine. These authors verified that disinfection was more effective in smoother surfaces. They suggested that in rougher surfaces, bacteria may hide and get protect from chlorine and thus have a higher chance to survive. The surfaces tested in the present work have similar surface energy and roughness, therefore, it seems that other parameters such as chemical composition or surface charge may have affected biofilm topology or the properties of the biofilm matrix resulting in a different biofilm sensitivity to chlorination.

4. Conclusions

In this work, it was shown that bacterial adhesion and biofilm formation on SICAN and stainless steel were similar, thus the surface coating was not able to prevent biological fouling. It was also shown that biofilm inactivation was higher in SICAN. An operational time window exceeding 6 hours was identified for the conditions used in this work where a higher hygienic status can be attained if SICAN is used instead of stainless steel. The duration of this time window and also the magnitude of this difference are likely to depend on the process conditions and need to be evaluated for each individual case. Also, if a satisfactory hygienic level is already attained with stainless steel, using SICAN may extend the operational time by reducing the frequency of cleaning or the duration of the cleaning period. The optimization of a cleaning schedule is not a trivial task (Pogiatzis, et al., 2014) but the same hygienic level may also be attained by lowering the concentration or the amount of cleaning agent. Additionally, there is also a potential for cleaning water saving. The implementation of these strategies will result in a more eco-friendly process.
particularly in food industries with frequent CIP operations (Table 1).

There has been some interest in the application of modified surfaces for biofilm reduction in the food industry, as the ability to modify operational parameters (flow rates, equipment geometries, temperatures,) is often limited (Gomes da Cruz, et al., 2015). However, due to the low profit margin of some products, there is often limited capital resources to replace existing materials or to produce equipment with modified surfaces which usually have a higher cost (Gomes da Cruz, et al., 2015). Therefore, the application of modified surfaces, would be more suitable for critical areas such as corners, valves or other types of areas which are shielded from the main flow where bacterial attachment is more likely to occur and where cleaning is particularly difficult. It is possible that the economic savings obtained from reducing water and disinfectants consumption or extension of the operational time may compensate for the capital investment necessary to use SICAN surfaces instead of stainless steel in critical areas of the process line.

Acknowledgments

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Application potential for the reduction of cleaning costs in the food processing industry.

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Figure captions

Figure 1 Attached cells at three time points (0.5, 2 and 6 h) in stainless steel (black bar) and SICAN (grey bar) surfaces at 5 ºC in: a) industrial water, b) industrial water with *E. coli* and c) medium with *E. coli*. Error bars shown for each surface, at each time point, represent the standard deviation from three independent experiments.

Figure 2 *E. coli* adhesion phase (until 6 h), biofilm growth phase (between 24 and 120 h), biofilm cleaning phase (between 120 and 121 h) and biofilm regrowth phase (between 121 and 144 h) in stainless steel (black line) and SICAN (grey line) surfaces. The interruption in the graph (between 6 and 24 h) represents the division between the results obtained for bacterial adhesion phase
assayed in microtiter plates and the biofilm assays performed in a flow cell. Both assays were made at the same shear stress (0.25 Pa), temperature (30 °C) and using the same culture medium. The disinfection time point is indicated with an arrow and the shaded area represents the time interval were a higher hygienic level can be achieved with SICAN. Error bars shown for each surface, at each time point, represent the standard deviation from three independent experiments.

Table 1 Typical cleaning times in the food industry

<table>
<thead>
<tr>
<th>Process/Industry</th>
<th>Cleaning Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salad washing</td>
<td>6-8 h</td>
</tr>
<tr>
<td>Milk pasteurization</td>
<td>4-8 h</td>
</tr>
<tr>
<td>Ice-cream production</td>
<td>24-62 h</td>
</tr>
<tr>
<td>Condiment industry</td>
<td>8-16 h</td>
</tr>
<tr>
<td>Artisan bread production</td>
<td>12-24h</td>
</tr>
<tr>
<td>Minced meat production</td>
<td>24 h</td>
</tr>
<tr>
<td>Meat preparation (cutting boards)</td>
<td>4 h</td>
</tr>
<tr>
<td>Refrigerated or RTE* frozen products</td>
<td>&lt; 24 h</td>
</tr>
<tr>
<td>Wine production</td>
<td>24 h</td>
</tr>
<tr>
<td>Beer production</td>
<td>36-120h</td>
</tr>
<tr>
<td>Beverage industry</td>
<td>60-100 h</td>
</tr>
</tbody>
</table>
* RTE – Ready To Eat. Cleaning schedule depends on the temperature of the room where the surface/equipment is located (from 4 h above 13°C up to 24 h for temperatures lower than 5°C)

Table 2 The apolar ($\gamma_{LW}^*$) and the surface polar parameters ($\gamma^+$ and $\gamma^-$), the hydrophobicity ($\Delta G$) and roughness of two surfaces (stainless steel and SICAN)

<table>
<thead>
<tr>
<th>Surface</th>
<th>$\gamma_{LW}$ / (mJ.m$^{-2}$)</th>
<th>$\gamma^+$ / (mJ.m$^{-2}$)</th>
<th>$\gamma^-$ / (mJ.m$^{-2}$)</th>
<th>$\Delta G$ / (mJ.m$^{-2}$)</th>
<th>Roughness$^a$/µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless Steel</td>
<td>36.8</td>
<td>0</td>
<td>8.90</td>
<td>-46.6</td>
<td>0.10±0.05</td>
</tr>
<tr>
<td>SICAN</td>
<td>36.1</td>
<td>0</td>
<td>6.52</td>
<td>-58.2</td>
<td>0.11±0.01</td>
</tr>
</tbody>
</table>

$^a$ values adapted from Boxler, et al. (2013a)
Figure 1 Attached cells at three time points (0.5, 2 and 6 h) in stainless steel (black bar) and SICAN (grey bar) surfaces at 5 °C in: a) industrial water, b) industrial water with *E. coli* and c) low nutrient medium with *E. coli*. Error bars shown for each surface, at each time point, represent the standard deviation from three independent experiments.
Figure 2 *E. coli* adhesion phase (until 6 h), biofilm growth phase (between 24 and 120 h), biofilm cleaning phase (between 120 and 121 h) and biofilm regrowth phase (between 121 and 144 h) in stainless steel (black line) and SICAN (grey line) surfaces. The interruption in the graph (between 6 and 24h) represents the division between the results obtained for bacterial adhesion phase assayed in microtiter plates and the biofilm assays performed in a flow cell. Both assays were made at the same shear stress (0.25 Pa), temperature (30 ºC) and using the same culture medium. The disinfection time point is indicated with an arrow and the shaded area represents the time interval were a higher hygienic level can be achieved with SICAN. Error bars shown for each surface, at each time point, represent the standard deviation from three independent experiments.

**Supplementary material:**
Figure S1 Bacterial adhesion (2 h) in SICAN and stainless steel at 30°C (black bars) and 5°C (grey bars).
Figure S2 Representation of the flow cell system used.